COX-2 EXPRESSION IN ENDOMETRIAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS

by

Dr PREETHI. M

A thesis submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY, CHENNAI

in partial fulfillment of the requirements for the award of the degree of

M.D in PATHOLOGY

DEPARTMENT OF PATHOLOGY

PSG INSTITUTE OF MEDICAL SCIENCE & RESEARCH

PEELAMEDU, COIMBATORE- 641 004

TAMILNADU, INDIA

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CERTIFICATE

This is to certify that the dissertation work entitled **"COX-2 expression in endometrial carcinoma and its correlation with clinicopathological parameters"** submitted by **Dr Preethi M,** is a work done by her during the period of study in this department from 30/05/2012 to 29/05/2015. This work was done under the guidance of **Dr.T.M.SubbaRao,** Professor, Department of Pathology, PSG IMS&R.

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CERTIFICATE

This is to certify that the thesis entitled **"COX-2 expression in endometrial carcinoma and its correlation with clinicopathological parameters"** submitted by **Dr Preethi M** to The Tamilnadu Dr MGR Medical University, Chennai, for the award of the degree of **Doctor of Medicine in Pathology,** is a bonafide record of research work carried out by her under my guidance. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Coimbatore – 641004
19.09.2014

Dr T M SubbaRao
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DECLARATION

I, Dr Preethi M , do hereby declare that the thesis entitled **"COX-2 expression in endometrial carcinoma and its correlation with clinicopathological parameters"** is a bonafide work done by me under the guidance of Dr T M SubbaRao, Professor in the Department of Pathology, PSG Institute of Medical Sciences & Research. This study was performed at the PSG Institute of Medical Sciences & Research, Coimbatore, under the aegis of the The Tamilnadu Dr MGR Medical University, Chennai, as part of the requirement for the award of the MD degree in Pathology.

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October 19, 2012

To
Dr M Preethi
I year Post Graduate
Department of Pathology
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Coimbatore

Ref.: Your study entitled 'COX 2 expression in endometrial carcinoma and its correlation with clinicopathological parameters'

Ref.2: Our letter dated 21.09.2012
Documents submitted by you on 12.10.2012

Sub.: Ethics Committee Approval for the study

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 21st September, 2012 in its expedited review meeting held at College Council Room, PSG IMS&R, between 9.00 am and 10.30 am, and discussed your application to conduct the study entitled:

"COX 2 expression in endometrial carcinoma and its correlation with clinicopathological parameters"

The following documents were received for review:

1. Duly filled application form
2. Confidentiality Statement
3. CV

After due consideration, the Committee has decided to approve the above study.

The members who attended the meeting held on 21.09.2012, at which your proposal was discussed, are listed below:

Name	Qualification	Responsibility in IHEC	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
Dr P Sathyan	DO, DNB	Clinician, Chairperson	Male	No	Yes
Dr S Bhuvaneshwari	M.D	Clinical Pharmacologist Member - Secretary	Female	Yes	Yes
Dr Sudha Ramalingam	M.D	Epidemiologist Alt. Member - Secretary	Female	Yes	Yes

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Dr Y S Sivan	Ph D	Member - Social Scientist	Male	Yes	Yes
Dr D Vijaya	Ph D	Member – Basic Scientist	Female	Yes	Yes

The approval is valid for one year.

We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R.

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Kindly note this approval is subject to ratification in the full board review meeting scheduled on 26.10.2012.

Yours truly,

19.10.12

SECRETARY
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INSTITUTIONAL HUMAN ETHICS COMMITTEE

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Endometrial cancer is the fifth most common cancer among women worldwide and the ninth commonest cancer among women in India [1]. It commonly occurs in postmenopausal women, with the median age being 63 years at presentation. Less than 5% of the endometrial cancers occur in women below 40 years of age [2]. It is the seventh leading cause of cancer related death among women [1].

I knew that writing a thesis was no child's play. Yet, all the work I had done, with as much enthusiasm as I could spare, resembled nothing more than a clay toy molded by the hands of a toddler, until it was perfected and given the right colors by my guide **Dr. T. M. SubbaRao**. I cannot thank him enough for all the time and energy he spared to perfect this document and I cannot admire him enough for the meticulous and critical way he handled each and every piece of information that we had gathered. Thank you very much sir!

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ABBREVIATION	EXPANSION
CD	Cluster of Differentiation
COX	Cyclooxygenase
ER	Estrogen Receptor
FIGO	International Federation of Gynecology and Obstetrics
ICD-O	International Classification of Diseases for Oncology
IHC	Immunohistochemistry
PG	Prostaglandin
PR	Progesterone Receptor
TXA_2	Thromboxane A_2
VEGF	Vascular Endothelial Growth Factor

TITLE OF THESIS: “COX-2 expression in endometrial carcinoma and its correlation with clinicopathological parameters”

ABSTRACT:

Endometrial cancer is a common cancer occurring in postmenopausal women. The important prognostic factors include FIGO stage, histologic grade, cell type, myometrial invasion and lymphovascular invasion. The advent of targeted therapy has led to a realm of studies on immunomarkers in various cancers including endometrial cancer. Cyclooxygenase-2 (COX-2) is one among the immunomarkers being studied in endometrial carcinoma.

We studied 37 uterine cancers diagnosed on hysterectomy specimens and found that 36 cases (97%) were positive for COX-2 and 29 showed overexpression (78%). Majority of the endometrioid carcinomas (76%) overexpressed COX-2. Overexpression was 100% in all the four non-endometrioid carcinomas, tumors with a nuclear grade of III and tumors presenting in stage II and above. Although the results were close to statistical significance, the observations are noteworthy.

KEY WORDS: Endometrial cancer, Cyclooxygenase-2 (COX-2), overexpression.

Endometrial cancer is the fifth most common cancer among women worldwide and the ninth commonest cancer among women in India [(1)]. It commonly occurs in postmenopausal women, with the median age being 63 years at presentation. Less than 5% of the endometrial cancers occur in women below 40 years of age [(2)]. It is the seventh leading cause of cancer related death among women [(1)].

Clinically, the smaller tumors are usually asymptomatic. The most common presentation is abnormal vaginal bleeding. Rare cases have been diagnosed incidentally from curetting or during an autopsy [(3)]. Pelvic pain, when present reflects spread of tumor [(2)]. The gold standard for diagnosis is histopathological study which can be established from tissues, obtained by endometrial curetting, hysteroscopic biopsy or hysterectomy specimens [(4)].

80% of endometrial cancers are of Type I and they are associated with conditions characterized by prolonged exposure to estrogens [(5)]. These include exogenous estrogens given for hormone replacement therapy, Tamoxifen therapy in breast cancer patients, increased endogenous estrogen exposure as in early menarche, late menopause, nulliparity, infertility, polycystic disease of ovary, obesity, diabetes and ovarian lesions associated with excessive and unopposed estrogen production as in granulosa cell tumor, thecoma, hyperthecosis and ovarian stromal hyperplasia [(2)].

Factors that influence the prognosis for endometrial cancers are FIGO stage, tumor grade, histologic type, myometrial invasion, lymph node status, vascular invasion and ER, PR status. The advent of targeted gene therapy has shifted the focus of research into studying the expression of various immunomarkers in endometrial cancers. Cyclooxygenase-2 (COX-2) is one amongst them.

COX-2 is an enzyme involved in the arachidonic acid pathway of inflammation. One of the end products of this pathway, the prostaglandins, has been thought to contribute to the inhibition of apoptosis and immune function, as well as the promotion of angiogenesis, invasion and metastasis[(6)].

Studies have shown that, the expression of COX-2 increases with the spectrum of endometrial disease, starting from simple hyperplasia and progressing to complex hyperplasia with atypia and endometrial cancer. Even within endometrial cancers, their expression increases with worsening histological grade [(7)]. Even the more aggressive squamous cell carcinomas have been found to show a more consistent association with COX-2 over expression [(8)].

We receive endometrial tissues of all these disease conditions. However, we have not studied expression of this immunomarker so far. Further, a literature search also did not reveal any publications on this immunomarker from South India. Hence, this study.

1. To identify the occurrence of endometrial cancer in the study period.
2. To observe the age distribution and the clinical stage at presentation of the endometrial cancers.
3. To observe the overexpression of the immunomarker COX-2 in the endometrial carcinoma tissues.
4. To correlate the cases with overexpression of COX-2 with the clinicopathological parameters such as menstrual status, parity index, FIGO stage, histological type, grade of the tumour and myometrial invasion.

UTERUS AND FALLOPIAN TUBES – A BRIEF OVERVIEW:

The uterus and the fallopian tubes are derived from the mullerian duct and are said to form a single structural & functional unit. Their prime functions during the reproductive phase of life are:

- Providing the site for fertilization
- Implantation of blastocyst
- Incubation of the embryo and fetus
- Delivery of the fetus at term [(9)].

They respond to the various hormonal stimuli as a single unit. Pathologically, the uterus, tubes and the ovarian surface epithelium form a single unit termed, the extended mullerian system and are affected by a similar group of neoplasms and metaplastic changes [(10)].

EMBRYOLOGY OF THE UTERUS AND FALLOPIAN TUBES:

The development of the male and female genital tracts takes place in two stages.

1. The indifferent stage
2. Specific sex differentiation (male or female)

1. THE INDIFFERENT STAGE:

In the indifferent stage, the precursors of the internal genital organs of both the sexes are laid down and they are similar to those of the bipotential gonad. Following this stage, female embryos lose the male anlage and vice versa. Both these anlages are closely related to the developing urinary system and so anomalies of the genital tract are often associated with those of the urinary system [(11)].

The mesonephric / Wolffian ducts and the urogenital sinus are well formed in the developing embryo by the sixth week. At this stage, the paramesonephric / mullerian ducts begin developing, by the invagination of the cealomic epithelium. The same also invests around the developing ovary. The distal ends of the mullerian ducts fuse in the midline and lose their medial walls resulting in a single cavity. This structure is called the uterovaginal primordium and it grows downwards towards the urogenital sinus [(9)].

2. SPECIFIC SEX DIFFERENTIATION (MALE OR FEMALE)

Sex differentiation depends on the gonad, whether it is the testis or the ovary. The developing testis in the male fetus, secretes testosterone which promotes the growth and differentiation of the Wolffian duct

into the male genital system. It also secretes antimullerian hormone, which causes the regression of the mullerian ducts. When there is no testosterone stimulation, the mullerian ducts persist and give rise to female genital organs. The uterus and upper third of vagina form from the fused end of the mullerian ducts while the fallopian tubes are formed from the unfused ends.

The uterus and vagina are fully developed by the 21st week of gestation. At this stage, the cervix is longer than the uterus. By birth, the uterus doubles in size, but the cervix remains larger in the prepubertal child. The homeobox gene family and the wingless gene family are said to be involved in these processes [(9)].

THE UTERUS IN THE PRE-PUBERTAL PERIOD:

At birth, the uterus measures about 4 cm. Its length is mostly constituted by the cervix. Maternal hormonal effect causes the newborn vagina to be thickened, with rugosity of the wall. The uterus grows in size up to two years of age and then stops till prepubertal changes start at the age of nine [(12)].

STRUCTURAL ANATOMY OF THE ADULT UTERUS:

1. LOCATION:

The uterus is a pelvic organ located behind the bladder and before the rectum. It is covered by peritoneum, which extends deeper in the posterior than the anterior aspect. Laterally, the peritoneum forms the broad ligament which ensheaths the fallopian tubes and the lymphatics and blood vessels of the uterus. The utero-ovarian ligament connects the ovary to the uterus. The other supports of the uterus are the cardinal ligaments, uterosacral ligaments and the pubocervical ligaments [(9)].

2. GROSS ANATOMY:

The uterus is a cavitated, pear shaped organ which is mostly muscular in nature. It measures approximately, 7x5x2.5cm. Its average weight is 40-80gms. Uterine measurements vary with age, menstrual cycle phase and parity. The cervix in the adult uterus is smaller than the corpus. The uterine corpus is divided into four parts:

i. Fundus

ii. Cornu

iii. Body

iv. Lower uterine segment

The fundus is located above an imaginary line connecting the origins of the tubes. The cornua are lateral to the fundus and this is where the intramural portions of the tubes lie. The body tapers downwards towards the lower uterine segment. The triangular uterine cavity is connected to the lumina of the fallopian tubes and the cervix. The approximate length of the cavity is 6 cm and it too varies with parity and age [(13)].

The cylindrical cervix has an approximate length of 3-4 cm . Part of the cervix is seen to protrude into the upper portion of the vagina called the portiovaginalis and the rest is seen above the vagina, called the portiosupravagnalis. It encloses the endocervical canal. The cavity opens into the vagina through the external os and the portion of the cervix upto the external os is called the ectocervix. The endocervical canal opens into the uterus through the internal os. Plicae palmatae are gross appearances of the mucosal clefts seen in the endocervix [(14)].

The uterus weighs more in a parous woman and the weight increases with parity. The vasculature is also more prominent than in nullipara. The cervical os, which is circular in the nullipara becomes slit-like in the

parous woman with an anterior and a posterior lip. The cervix in parous women may also show healed lacerations and ectocervical 'erosions' that are caused by the presence of endocervical mucosa outside the external os.

In postmenopausal women, the uterus decreases in size and weight. Sometimes the endocervical canal may even become obliterated. Hormone replacement therapy may delay these effects to some extent [(9)].

3. BLOOD SUPPLY:

The right and left uterine arteries supply the uterus. They branch at the isthumus into ascending and descending arteries. The ascending branches extend upward and anastamose with the arterial supply of the ovaries and the descending branches anastamose with those of the vagina. The arcuate arteries are formed in the subserosa from the ascending and descending arteries which in turn give rise to the radial arteries which penetrate through the myometrium. These in turn give rise to the straight arteries supplying the basal region of the endometrium and the spiral arteries which supply the rest of the endometrium [(9)].

4. **LYMPHATIC DRAINAGE:**

The lymphatic channels are seen close to the endometrial glands in the mucosa. In the myometrium and cervical stroma, the lymphatic channels of

both these regions often anastamose and reach the subserosal plexus. The draining nodes start at the parametrium and paracervical region and, go on to the inguinal nodes, obturator set of nodes, the hypogastric nodes, the external and common iliac nodes, the sacral nodes and finally into the aortic nodes [(15).]

HORMONES THAT ACT ON THE UTERUS:

At birth, secretion of endogenous hormones does not take place. However, estrogen produced by the mother may influence the uterine endometrium and the cervix transiently [(12).]

At puberty, the endogenous hormone secretion begins but is quite unpredictable for the first few years, resulting in anovulation and highly variable cycle lengths [(16).]

The regular menstrual cycle requires the participation of four different organs:

1) The hypothalamus, which secretes the gonadotrophin releasing hormone (GnRH).
2) The pituitary, which secretes follicle stimulating hormone (FSH) and luteinizing hormone (LH).
3) The ovarian cortex, which produces estrogen and progesterone.

4) Endometrium, which responds to these steroid hormones.

In the hypothalamus, the arcuate nucleus, releases GnRH in a pulsatile manner. This GnRH, controls the release of the pituitary hormones. FSH stimulates the secretion of estrogen by the ovarian follicles and LH, the secretion of progesterone by the corpus luteum. These in turn, suppress the secretion of the pituitary hormones by a negative feedback mechanism [(17).]

FOLLICULAR/PROLIFERATIVE PHASE:

Prior to the starting of the menstrual period, the production of FSH increases. This causes the recruitment of follicles in the ovarian cortex. One of these follicles becomes dominant and converts circulating androgens into estrogens, mainly estradiol, in the presence of LH [(18).] Regulatory hormones, namely, activins and inhibins are also secreted by the ovarian follicles. Inhibins suppress FSH production, whereas, activins increase the follicular response to FSH. Proliferative phase lasts for the first 2 weeks of the menstrual cycle [(19).]

SECRETARY/LUTEAL PHASE:

Ovulation occurs as a result of a midcycle peak in the production of LH and estradiol [(18)]. This is followed by the production of progesterone, in the corpus luteum, by the granulosa cells. Progesterone in turn causes secretory

changes in the endometrium. During secretary phase, estrogen production persists at a lower level [17].

MENSTRUAL PHASE:

When fertilization fails to occur, the corpus luteum regresses and the highly vascular predecidualized endometrium undergoes disintegration and is shed over a period of five days. By the end of the menstrual phase, only the stratum basalis remains. As the next cycle begins, the epithelium from the gland crypts proliferate and the stromal integrity is regained [17].

The gestational period is characterized by an increased production of progesterone which sustains the pregnancy [20].

Around the fifth decade of life, the hormonal secretion begins to wane and results again in irregularity of the cycle lengths. Finally, as there are no more maturing follicles in the ovary, ovulation stops and so does the menstrual cycle [21].

HISTOLOGY OF THE CERVIX

The two portions of the cervix namely, the ectocervix and the endocervix are lined by different epithelia. The ectocervix is lined by a stratified squamous epithelium which is of a non-keratinized type. In the reproductive

years, this epithelium is composed of three layers- the basal/parabasal, the intermediate and the superficial. There is also an increase in accumulation of glycogen in the more superficial cells. The cervix at birth may show all three layers and even glycogenation of the squamous cells, due to the maternal hormonal effects.

After menopause, the epithelium only shows the basal/parabasal layer and there is also an absence of glycogen within the cytoplasm of these cells. There is also an increase in nuclear:cytoplasmic ratio, which may be confused with a cervical intraepithelial neoplasm [(22)].

The lining of the endocervix is composed of mucin secreting glandular cells with basally placed nuclei, arranged in a single layer. At birth, the endocervical epithelium is often seen on the ectocervix, but it soon reverts to its position at the external os. After puberty, this layer may extend into a portion of the anatomical ectocervix as well, in the form of "erosions" due to swelling of the cervix.

The transformation zone is situated between the endocervical glandular epithelium and the squamous epithelium. In the foci of "erosions", the glandular epithelium gets replaced by metaplastic squamous cells, altering the original squamo-columnar junction. The transformation zone begins to

get pulled up again in the late reproductive years and by the time menopause occurs, the squamo-columnar junction is found well within the endocervical canal.

During pregnancy, the endocervical epithelium shows proliferation to increase the production of mucin.

The cervical stroma is vascular and is composed predominantly of fibrous tissue with a few smooth muscle fibers running in between [(9)].

HISTOLOGY OF THE UTERUS

Histologically, the uterus shows three layers- the innermost endometrium, the myometrium and the serosa.

THE ENDOMETRIUM:

The endometrium is composed of glands lined by columnar epithelium (which varies in appearance in accordance with the phase of the menstrual cycle) and stroma which is composed of elongated endometrial stromal cells, lymphoid infiltrate, a reticulin framework and vascular elements.

At birth, the endometrium might be proliferative or even weakly secretory for a transient period due to the maternal estrogen influence.

Histopathologic phases of the menstrual cycle:

Menstrual phase

Early proliferative and proliferative phase

Day-16 endometrium

Secretory phase - vacuolated, exhaustive and predecidual [17].

Menstrual phase endometrium:

In this phase there is diffuse breakdown of the endometrial stroma and the stratum functionalis is shed leading to the menstrual bleeding. Progesterone levels drop and matrix metalloproteinases such as gelatinase, collagenase, stomolysins etc., are released along with clotting factor activation followed by fibrinolysis [23].

The earliest morphologic change that occurs is predecidual condensation into cords and groups separated by blood and inflammatory cells. Secretory exhausted glands are seen, which may be large and irregular with slight nuclear stratification to delicate, thin, single layered epithelial strands. The coordinated collapse of the functionalis with necrotic predecidua and sheets of neutrophils is called “scheduled breakdown”. An ovulatory cycle can be

most often diagnosed in the presence of predecidualised stroma and exhausted glands [24].

When corpus luteum persists, irregular shedding occurs in which case the menstrual phase endometrium shows features of late secretory phase, like predecidualisation, along with late proliferatory phase changes like star shaped glands. Membranous dysmenorrhoea is a painful condition where the endometrium is shed in the form of a cast of the uterine corpus [17].

Early proliferative and proliferative phase:

In this phase, the glands appear tubular; the nuclei show pseudostratification and increased mitosis. Nuclear chromatin may appear coarse and the increased mitosis may give rise to a suspicion of malignancy. The nuclear size is regular and the glands are rounded with minimal irregularity and thus it is easily distinguished from malignant glands.

Just before ovulation, edema develops in the stroma and the glands become tortuous. Confusion with secretory phase may occur when there is prominent spindling of cells, but there are no spiral arterioles. Also presence of ‘secretions’ in glandular lumina does not indicate secretory phase when other changes are not present [17].

Day 16:

Subnuclear vacuoles start appearing and mitotic rate is decreased. Other findings are that of a proliferative phase endometrium [(24)].

Vacuole phase: 17-19 days

Vacuoles are present uniformly in all glands and no mitosis is seen. If mitosis is seen with the above findings, a diagnosis of an ovulatory cycle cannot be made. In day 17 endometrium, vacuoles are subnuclear, pushing up the nuclei. In day 18, vacuoles move towards the lumen & secretary material starts appearing in the lumen. In day 19, the nuclei become basal again and the glands have a secretory exhausted appearance [(17)].

Exhaustive phase: 20-22 days

Secretory exhaustion is seen. This is followed by edema which increases and peaks by day 22 with prominence of spiral arterioles [(17)].

Predecidual phase: 23-28 days

Spiral arterioles are surrounded by predecidualized stroma i.e spindled cells occurring in a spiral aggregate around vessels. The stroma below the epithelium gets predecidualized next. Then there is confluence of

predecidua below the epithelium and between glands. Finally stromal granulocytes appear and become prominent [17].

Arias-Stella reaction

Arias-Stella first reported this endometrial epithelial change to occur as a result of chorionic hormonal stimulation. He emphasized on the fact that, this reaction can be diagnosed only in the presence of nuclear enlargement and not just cellular enlargement which can occur in a hypersecretory endometrium. The enlarged nuclei may be vesicular, hyperchromatic or even pyknotic looking, with irregularity of the nuclear membrane. Other changes that can be seen are chromatin clearing and nuclear pseudoinclusions. Because of these nuclear changes, this condition was thought to be an early malignancy before his report was published. This change is most often patchy and may be seen in uterine and extrauterine pregnancies, trophoblastic tumors and in post-abortion or post-partum uteri [20].

The atrophic endometrium

After menopause, the lining epithelium of the endometrium becomes flattened to cuboidal with loss of cilia. The glands may become cystically

dilated. The stroma shows spindle cells which do not undergo predecidualization [25].

THE MYOMETRIUM:

The uterine myometrium has an inner circular layer which extends to surround the tubal lumina and the cervical canal. The middle layer is composed of highly vascularized, interdigitating bundles of muscle fibers. The outermost layer is composed of longitudinal muscle bundles. During pregnancy the uterus increases in size owing to the hypertrophy as well as hyperplasia of the uterine muscle fibers. There is also an increase in the extracellular matrix and the vasculature. Post pregnancy, the uterus deceases in size owing to a decrease in size of the individual myocytes [9].

DISEASES OF THE CERVIX: AN OVERVIEW

- Metaplasias: Squamous, tubal, tuboendometrioid and transitional cell
- Inflammatory conditions: Non infectious cervicitis- may result from erosion, iatrogenic injury- biopsy or conization or radiation.
- Infections – Cervicitis: The commonest infections are those caused by Candida species, Trichomonas vaginalis, Neisseria gonorrhoeae, Gardnerella vaginalis and Herpes simplex virus (HSV). Other organisms causing infections are, Chlamydia trachomatis, Treponema pallidum,

- Mycobacterium tuberculosis, Actinomyces, Human papilloma virus (HPV), Cytomegalovirus (CMV) and Schistosoma hematobium.These conditions can cause reactive atypia and/or hyperkeratosis and parakeratosis.
- Cervicovaginitis emphysematosa: This condition is characterized by multiple subepithelial cysts which are filled with gases including carbon monoxide. These spaces are not lined by any epithelium and do not seem to be caused by any gas forming bacteria.
- Pseudoneoplastic conditions: Microglandular endocervical hyperplasia, diffuse laminar endocervical glandular hyperplasia, lobular endocervical glandular hyperplasia, endocervicosis, endometriosis, mesonephric hyperplasia and remnants, decidual pseudopolyps, inflammatory pseudotumor and mullerian papilloma.
- Benign neoplasms: Endocervical and mesodermal stromal polyps, leiomyoma, adenomyoma, papillary adenofibroma, placental site trophoblastic nodule, hemangiomas, neurofibromas and ganglioneuromas, nevi and cysts such as nabothian cyst, inclusion cyst and tunnel clusters.
- Precancerous conditions: cervical intraepithelial neoplasia- squamous and glandular.

- Malignant tumors:

  i) From the squamous epithelium- squamous cell, verrucous, warty, transitional and lymphoepithelioma-like carcinomas.

  ii) From the glandular epithelium- adenocarcinoma-NOS, villoglandular, clear cell, serous and mesonephric adenocarcinomas.

  iii) Others- adenosquamous carcinoma, glassy cell carcinoma, neuroendocrine tumors and salivary gland type malignancies- adenoid cystic, mucoepidermoid and adenoid basal carcinomas.

  iv) Mixed epithelial and mesenchymal tumors and secondaries [(26)].

DISEASES OF THE UTERUS – AN OVERVIEW

- Inflammations: These could be non infectious and infectious. Infectious causes include Chlamydia, Mycoplasma, Actinomyces, CMV, HSV, fungi and parasites. Non infectious causes include ligneous endometritis, malakoplakia, lymphoma-like lesions, dysfunctional uterine bleeding, epithelial metaplasias (squamous, tubal, mucinous, clear cell, hobnail, oncocytic and papillary syncitial), mesenchymal metaplasias (smooth muscle, glial, adipose, cartillagenous and osseous), extramedullary hematopoiesis,

Asherman's syndrome, effects of IUCD, emphysemoatous endometritis, post-operative spindle cell nodule etc.

- Benign neoplasms: Endometrial polyps, atypical polypoid adenomyoma, adenofibroma etc.
- Precancerous conditions: Endometrial hyperplasias- simple hyperplasia without atypia, simple hyperplasia with atypia, complex hyperplasia without atypia, complex hyperplasia with atypia, hyperplasias with metaplasia (squamous, tubal, secretory, hobnail, mucinous and mixed cellular) and endometrial intra-epithelial carcinoma.
- Cancers:

i) Endometrioid adenocarcinomas, non-endometrioid adenocarcinomas, carcinosarcomas, germ cell tumors and metastatic tumors.

ii) Mesenchymal tumors: Leiomyoma, diffuse leiomyomatosis, dissecting leiomyoma, intravenous leiomyoma, benign metastasizing leiomyoma, peritoneal (parasitic) leiomyoma, leiomyosarcoma, smooth muscle tumor of uncertain malignant potential (STUMP), perivascular epithelioid cell tumor, stromal tumors- benign and malignant, rhabdomyosarcoma, alveolar soft

iii) part sarcoma, adenomatoid tumor and primitive neuroectodermal tumor [26].

THE WHO CLASSIFICATION OF ENDOMETRIAL CARCINOMAS:

1. Endometrioid adenocarcinomas:
    i. With squamous differentiation
    ii. Villoglandular variant
    iii. Secretory variant
    iv. Ciliated cell variant
2. Non endometrioid adenocarcinomas-
    i. Mucinous
    ii. Serous
    iii. Clear cell
    iv. Mixed cell
    v. Squamous cell
    vi. Transitional cell
    vii. Small cell
    viii. Undifferentiated [27].

ENDOMETRIAL CARCINOMA

INTRODUCTION:

About 3.2 lakh cases of cancer of uterus were reported in the year 2012, globally. The incidence of the cancer of the corpus uteri among women (globally) is 4.8%. In India, the incidence is only 2.3% and accounted for 1.5% of cancer related mortality [(1)].

Carcinomas of the endometrium are much more common than malignant mesenchymal neoplasms of the uterine corpus. The most common histologic type is the endometrioid adenocarcinoma (80%).The non endometrioid carcinomas comprise about 20% [(28)]. Serous (5-10%) and clear cell (1-5%) are more frequent than the other non endometrioid carcinomas [(27)].

CLINICAL FEATURES:

Endometrial carcinomas are most common in the postmenopausal age group with a median age of 63 years. Less than one percent cases occur below the age of 40 years [(2)]. Most cancers occurring in this younger age population are endometrioid adenocarcinomas of a better differentiation and present at an earlier stage compared to those occurring in postmenopausal women [(28)]. Occasional cases have been diagnosed in pregnant women.

The commonest symptom is abnormal vaginal discharge most commonly abnormal vaginal bleeding, with a few cases presenting with leucorrhoea. In advanced cases with invasion, pelvic pain, pressure symptoms and metastasis related symptoms are seen.

GROSS PATHOLOGY:

Grossly, the tumor might be single, multiple or diffusely involving the endometrium. Most commonly, it appears as a broad based polypoidal mass with an irregular surface. Diffuse infiltration causes a bulky uterus. Minimal deviation invasion is the term used, when a very deep myometrial invasion has occurred without significant increase in size of the uterus. Younger patients have lesions more towards the lower uterine segment [(28)].

HISTOPATHOLOGY:

Endometrioid Adenocarcinoma:

The tumor is composed of glands arranged in a complex cribriform pattern often with maze like interconnected lumina, solid areas and villoglandular pattern. The cells look similar to proliferative phase cells and show multilayering. The cells are larger than those seen in normal endometrium with rounded nuclei, clumped chromatin and often show prominent nucleoli. There may be small foci showing squamous, mucinous, tubal or other cell

types. Mitotic figure numbers vary with differentiation of the tumor. Stromal invasion within endometrium does not often provoke desmoplastic reaction, whereas spread into the muscle is often surrounded by a desmoplastic reaction of the stroma. Stroma may show some foamy cells.

The criteria to diagnose malignancy include:

1) Cribriform glands
2) Solid areas
3) Interconnected lumen
4) Irregular glands

Variants:

i) With squamous differentiation: Squamous component is present to some extent in one fourth of endometrioid carcinomas. They are both neoplastic and metaplastic. When the squamous component is well differentiated, it is called adenoacanthoma. When poorly differentiated it is known as adenosquamous carcinoma. The former has a better prognosis.

ii) Secretory cell carcinoma: It is an uncommon variant where the neoplastic glands show secretory changes, especially supranuclear

iii) and/or subnuclear vacuoles. The changes may be progestin induced or it may be an intrinsic feature of the malignant cells. Sheets of cells with clear cytoplasm but not with significant atypia as seen in clear cell carcinoma may also be seen. The prognosis is good.

iv) Ciliated cell carcinoma: It is a rare variant. Occasional ciliated cells may be seen in most cases. If more than 75% cells have cilia, a diagnosis of ciliated cell carcinoma can be made. There also needs to be invasion for it to be differentiated from endometrial intraepithelial neoplasia. It has a good prognosis.

v) Villoglandular variant: This has, long, slender papillae with cytological features of grade I endometrioid adenocarcinoma. Psammoma bodies, usually seen in serous carcinomas, may be seen in these tumors as well. The prognosis is excellent.

<u>Differentiating well differentiated endometrioid carcinoma from complex hyperplasia with atypia:</u>

Histologically, a complex cribriform glandular pattern in more than 30% of the biopsy tissue or marked nuclear atypia with pleomorphism and prominent nucleoli, are features more indicative of malignancy.

Myoinfiltration seen in hysterectomy specimens is the only conclusive evidence of malignancy. Genetically, PTEN and/or P53 mutation and a clonal expansion in tumor cell are signs of malignancy.

Mucinous Adenocarcinomas:

This accounts for a small percentage of endometrial carcinomas. It is often seen associated with an endometrioid component. It is more common in patients who have received Tamoxifen treatment or exogenous progestins. Presence of intracytoplasmic mucin may be seen in some of the cells in endometrioid adenocarcinoma. When more than half of the cells show this feature, the tumor is classified as mucinous adenocarcinoma. In biopsy specimens, they look a lot like endocervical fragments, but the delicate stroma, microglandular component and the alternation with non- mucinous endometrioid type fragments is often diagnostic. The prognosis is usually good.

Serous Carcinoma:

They are aggressive type tumors with an older age of incidence than endometrioid type carcinomas. There is often no history of exposure to excessive estrogens, whether intrinsic or extrinsic and the women are mostly parous with normal serum estrogen levels. Endometrial hyperplasias or

intraepithelial neoplasia have not been associated with this tumor, but serous intraepithelial carcinoma and endometrial glandular dysplasia have been identified as its precursor lesions. These tumors show complex, branching papillae with thick fibrovascular cores. Tufting of lining cells may be seen. There may also be glandular and solid areas. The lining cells are usually polygonal with marked pleomorphism, high N:C ratio and macro nucleoli. Tumor giant cells can be seen. Exfoliative cells with hob nailing are common. The prognosis is very poor.

Squamous Cell Carcinoma:

They are rare and occur at an older age group compared to other endometrial carcinomas. Icthyosis uteri, a condition in which there is extensive squamous metaplasia of the endometrium, is considered to be its precursor. It resembles squamous cell carcinomas in other organs and has little or no adenocarcinomatous elements. The prognosis is extremely poor with a median survival of only 9 months.

Clear Cell Adenocarcinoma:

A small percentage of endometrial carcinomas have this morphology. They occur at an older age than the endometrioid adenocarcinomas. The cells may be arranged in a papillary, solid, glandular or tubular-cystic pattern. Most of

the cells are seen to have a clear cytoplasm. Mitosis is frequently noted. Some of the cells have scant cytoplasm and large nuclei protruding into the lumen in a hobnail appearance. Prognosis is poor.

Mixed Carcinoma:

The term mixed carcinoma can be used when the individual components exceed 10% of the tumor volume.

Undifferentiated Carcinoma:

These tumors can be identified to be of epithelial origin, but further sub-classification cannot be done. The cells may be large, anaplastic and form vague glandular pattern, or they may be of a small cell type [(2)].

Transitional Cell Carcinoma:

It usually shows papillary arrangement of cells. Often there is an associated second tumor type which may be endometrioid, clear cell or serous. This tumor may show koilocytes which are associated with HPV infection [(27)].

TECHNIQUES TO OBTAIN AN ENDOMETRIAL SAMPLE FOR PATHOLOGICAL STUDY:

1. **DILATATION AND CURETTAGE**

Endometrial samples can be obtained by dilatation and curettage, but it is a procedure with much discomfort to the patient. It may still miss 5-10% of endometrial lesions.

2. **ENDOMETRIAL BIOPSY**

Limited biopsies can be obtained without dilatation, as an office procedure, with much less discomfort to the patient. When guided by hysteroscopy, a representative biopsy can easily be obtained.

3. **VEBRA ASPIRATOR BIOPSY**

This method uses a suction curettage device called the Vebra aspirator, which is made of steel. It is preferred for women of reproductive age group as it often provides inadequate samples in postmenopausal women.

4. **PIPELLE BIOPSY**

The device used for this biopsy is widely used in the United States and in Europe and is performed as an office procedure. Pipelle is also a suction curettage device, but is made of plastic [(2)].

5. ENDOMETRIAL CYTOLOGY

Sample can be obtained using a Tao brush, which is an ensheathed brush. This can be introduced up to the uterine fundus. The sheath is then removed. The exposed bristles will collect the endometrial cells when rotated in one direction only for four to five times. The sheath is then replaced to cover the bristles to avoid contamination with cervical or vaginal material, while taking the brush out. This brush is then cut off into a container with liquid fixative and processed as for cervical liquid based cytology. The advantage with this procedure is that it is relatively painless [(29)].

6. HYSTEROSCOPIC BIOPSY

This allows for a targeted biopsy of the hysteroscopically abnormal region of the endometrium [(2)].

RISK FACTORS FOR ENDOMETRIAL CARCINOMA

Excessive estrogen stimulation is the most common risk factor and it may be exogenous or endogenous.

Exogenous estrogen stimulation is seen with Tamoxifen, administered to breast cancer patients in whom it acts as a competitive inhibitor of estrogens with an antagonistic effect. In the endometrium though, it seems to have a

weak agonistic effect, especially in postmenopausal women. The increase in risk is 2-3 times and the tumors are more often low grade [30].

Endogenous estrogen exposure may be physiological or pathological.

Physiological causes include, early age at menarche, late menopause and low or nulliparity.

Pathological causes include infertility, anovulatory cycles and estrogen producing ovarian conditions like granulosa cell tumors, thecomas, polycystic ovarian disease and hyperthecosis [2].

Other associated risk factors are obesity, diabetes, gonadal dysgenesis, Stein-Leventhal syndrome and Hereditary Non Polyposis Colonic Cancer (HNPCC) syndrome [22].

Cigarette smoking has been shown to have a protective effect in post menopausal women who are current smokers [31]. Conversely, smoking has been associated with a more advanced stage at presentation [32].

FACTORS THAT INFLUENCE THE PROGNOSIS IN ENDOMETRIAL CARCINOMA

Prognostic factors help in establishing the probable disease outcome for a patient and also to determine the need for adjuvant therapy. A useful prognosticator ought to:

1) Help identify two or more sets of patients on the basis of disease free survival and recurrence.
2) Both univariate and multivariate analysis must show it to have a statistical significance as a prognosticator.
3) The population studied must be large and representative.
4) It should be reproducible.
5) Multiple studies must have proved its significance.

The important prognostic factors for endometrial carcinomas include:

1) FIGO stage
2) Histologic grade
3) Cell type
4) Myometrial invasion

5) Vascular and lymphatic invasion

6) Peritoneal cytology

7) Ploidy

8) Steroid receptors

9) Bcl2 and proliferation markers

10) Angiogenesis and VEGF

1. FIGO STAGING OF ENDOMETRIAL CARCINOMA:

The International Federation of Gynecology and Obstetrics (FIGO) adopted its first rules for the staging of uterine cancer in 1958. The UICC (International Union Against Cancer) and the American Joint Commission on Cancer (AJCC) published their own staging systems in 1966 and 1976 respectively. FIGO revised its staging system subsequently and the 1988 revision was in vogue till September 2008, when the revised proposal of FIGO staging for endometrial cancer was approved by the FIGO executive board. What follows below **(*Table 1*)** is the Revised FIGO staging published in 2009 in the International Journal of Gynecology and Obstetrics, which is the official mouthpiece of FIGO[33]. Multivariate studies have proved it to be the single strongest predictor of outcome for endometrial carcinomas [22].

Table 1: Revised FIGO staging of endometrial carcinoma

STAGE	CHARACTERISTICS
I A	Tumor involving the endometrium with no or <1/2 of myometrium involved
I B	Tumor involving the endometrium with >1/2 of myometrium involved
II	Cervical stromal involvement
III A	Serosa of the uterus or adnexae involved
III B	Vaginal or parametrial involvement
III C	Metastasis to pelvic or para-aortic nodes C1- pelvic nodes involved C2- para-aortic nodes involved with/without pelvic node involvement
IV A	Invasion of bladder and/or bowel mucosa
IV B	Distant metastasis, including intra-abdominal and/or inguinal nodes

Endometrioid adenocarcinomas usually present at stage I, whereas serous carcinomas present at stage III or IV in 72% of cases [(2)].

2. HISTOLOGIC GRADING:

There are two systems of histological grading, one based on the tumor architecture **(*Table 2*)** and the other based on the nuclear morphology **(*Table 3*).**

Table 2: Grading of endometrial carcinoma based on tumor architecture:

GRADE	CHARACTERISTICS
Grade I	</= 5% tumor growth is in solid sheets
Grade II	6-50% tumor growth is in solid sheets
Grade III	>50% tumor growth is in solid sheets

Architectural grading does not take into account areas of squamous differentiation [(22)]. The most common architectural grade of endometrioid adenocarcinoma is grade I (50%). Grade 3 cancers are often associated with an older age at presentation [(28)].

Table 3: Grading of endometrial carcinoma based on nuclear features:

GRADE	CHARACTERISTICS
Grade I	Round to oval nuclei with evenly distributed chromatin and inconspicuous nucleoli
Grade II	Irregular oval nuclei with chromatin clumping and moderate sized nucleoli
Grade III	Large pleomorphic nuclei with coarse chromatin and large irregular nucleoli

When the nuclear grade is three, the architectural grade of grade I and II lesions is raised by one for endometrioid adenocarcinomas. Generally, while grading the non-endometrioid adenocarcinoma, only the nuclear grade is used except in mucinous carcinoma where architectural grading is preferred [(22)].

3. CELL TYPE:

Endometrioid carcinoma, its variants and mucinous adenocarcinoma have a good prognosis and are often classified as type I endometrial carcinoma. Whereas, serous adenocarcinoma, clear cell adenocarcinoma and squamous cell carcinoma have a poor prognosis and are clubbed under type II endometrial carcinoma [(22)].

4. MYOMETRIAL INVASION:

Myometrial invasion may be absent or present. If present, there may be invasion up to the inner half or the invasion may extend into the outer half. The prognosis worsens in proportion with the depth of invasion. Involvement of adenomyotic sites cannot be considered as myometrial invasion. There needs to be a desmoplastic reaction around the malignant glands to call it a true invasion [(22)].

5. VASCULAR AND LYMPHATIC INVASION:

Invasion into vascular or lymphatic channels is diagnosed by the presence of nests of cells surrounded by flattened endothelial lining cells. The recurrence risk is higher when invasion is present. Lymphatic invasion in particular is an independent prognostic factor with poor survival and high recurrence rate [(22)].

6. PERITONEAL CYTOLOGY:

The only indicator of extra uterine spread in about 5-15% cases is the presence of positive peritoneal cytology. The recurrence rate was found to be 40% as compared to 10% in patients with a negative peritoneal cytology. It predicts an increase in disease related death [(22)].

7. PLOIDY:

Flow cytometry and static cytometry can help determine the ploidy of cells. In most endometrial carcinomas around two thirds of cells have been found to be diploid. Tumors with diploid cells as a majority have a better prognosis than those with more of aneuploid cells [(22)].

8. STEROID RECEPTOR STATUS:

Estrogen and progesterone receptor positivity indicates a better differentiation of the tumor, better prognosis with less recurrence and better survival [(22)].

9. PROLIFERATION MARKERS:

The proliferation rate of cells within the tumor can be assessed by mitotic counts, S-phase fraction determination using flow cytometry and immunohistochemical staining for proliferating cell nuclear antigen. Increase in proliferation rate indicates a progressing tumor and so a poorer prognosis [(22)].

10. APOPTOSIS MARKERS:

Bcl2 expression has been found to increase from endometrial hyperplasias to poorly differentiated carcinomas. Decreased Bcl2 expression correlates with PR negative status, increased depth of invasion, increased FIGO stage, worse cell types, increased lymph node metastasis and tumor recurrence [(22)].

11. ANGIOGENESIS AND VEGF:

Proliferation of capillaries is essential for continued tumor growth. VEGF expression is an independent prognostic factor for poor survival, poorer differentiation, increased stage and lymphatic invasion [22].

ROLE OF IMMUNOMARKERS IN ENDOMETRIAL CARCINOMA

IMMUNOMARKERS- A BRIEF HISTORY

Albert H. Coons first developed immumomarkers for an immunofluorescence method for detection of specific antigens in frozen sections, in the year 1940 [34]. Later, the horseradish peroxidase was used to label the antibodies, along with a color producing substrate, so that the immunomarkers can be used in light microscopic detection of antigens [35]. The sensitivity of the procedure was further increased by making it a multistep process using avidin-biotin conjugate, biotin streptavidin method, amplification using substances like tyramide and polymer based labeling system [36].

Initially, the technique had application only with cryostat preparations, but in 1974, it was shown that it could be applied to paraffin-embedded tissues

as well, by Taylor and his collegues [37]. In the year 1991, the antigen retrieval technique, which involves heating of paraffin sections, was developed to unmask antigens that have become altered by fixation in formalin. This enhanced the intensity of staining by the immunomarkers [38].

IMMUNOHISTOCHEMISTRY (IHC) – BASIC CONCEPTS

It is a technique based on the recognition of specific antigens by their antibodies, and by this interaction, these antigens can be located on their specific sites in the cells and tissues.

An antigen, also known as an epitope, is a molecule which can stimulate the production of specific antibodies, and which possesses one or more sites for the binding of that antibody.

An antibody is a protein substance with one or more of a basic unit called the monomer which is Y shaped. This monomer has a pair of light chains and a pair of heavy chains. The Fab portion- formed by the two light chains and portions of the two heavy chains- is the portion that binds with the antigen. The remainder of the heavy chain regions forms the Fc (fragment crystallizable) portion of the antibody. Monoclonal and polyclonal antibodies are being produced commercially. Polyclonal antibodies are

composed of a mixture of antibodies, which can detect several epitopes on a single antigen. These are produced using live animals and can be used on denatured proteins as well as antigens that have lost one or a few epitopes. Monoclonal antibodies, on the other hand, can detect only a single epitope

on an antigen. Though it is highly specific, it is sensitive to loss of a single epitope on the antigen. These are produced from cell cultures [(39)].

Advantages of immunohistochemistry:

- It can be done on formalin fixed paraffin embedded specimens.
- It can be done on stored, archival material
- It is highly sensitive and specific
- When used on frozen section, an easily reproducible immunofluorescence technique which is simple and rapid can be used.
- When used on paraffin blocks, peroxidase in combination with a diaminobenzidine chromogen allows for long term storage of slides without fading of the stain
- There is no need for a high cost equipment like a fluorescence microscope[(39)].

IMMUNOHISTOCHEMISTRY –GENERAL APPLICATIONS

The main application of IHC is in the typing of the cellular differentiation of neoplasms, which may in many instances be challenging on light microscopy. Earlier, electron microscopy was used for this purpose, but nowadays, IHC has replaced this costly and tedious procedure.

This has been especially useful in the workup of:

- Anaplastic tumors
- Small round cell tumors
- Soft tissue tumors
- Lymphoma
- Metastatic tumors with an unknown primary

In some instances it has also been used to differentiate a reactive process from a neoplasm- e.g. differentiation of a low grade astrocytoma from a reactive gliosis.

They are also used as prognostic indicators- e.g. proliferation markers like MIB-1 can demonstrate the aggressiveness of tumors and gene amplification of oncogenes like HER2/neu indicates a bad prognosis.

Some markers like the estrogen and progesterone receptors are used to predict the responsiveness of a tumor to therapy.

Detection of infectious viruses such as HSV, CMV, oncogenic viruses such as HPV, HSV, Estein Barr virus (EBV) and other infectious agents such as Mycobacteria, Cryptococcus etc., has also been made using immunomarkers.

The diagnosis of some non-neoplastic diseases of the brain and the muscle also requires immunomarkers [39].

IMMUNOMARKERS THAT HAVE BEEN STUDIED IN ENDOMETRIAL CARCINOMA:

Advanced type I and type II cancers have a poor response to therapy and a worse prognosis. These are the tumors that may with the help of novel therapeutic targets be treated more adequately through individualized treatment.

Changes in gene expressions induced by mutations can be studied using IHC and thus newer therapeutic targets can be identified. Many are underway in which various targeted therapies are being used as monotherapy or in combination with chemotherapy and radiotherapy. Majority of these studies use receptor tyrosine kinase (RTK) inhibitors,

mammalian Target of Rapamycin (mTOR) inhibitor and estrogen inhibitors like Selective Estrogen Receptor Modulators (SERMs) and aromatase inhibitors. The remaining trials use agents like cyclooxygenase-2 (COX-2) inhibitors, cell cycle inhibitors, gonadotropin releasing hormone (GnRH) analogues, folic acid analogs, histone deacetylase (HDAC) inhibitors, the

monoclonal antibody RAV12, CALAA-01, protein kinase-C inhibitors and mitogen activated protein kinase (MAPK) inhibitors.

ErbB receptor family and vascular endothelial growth factor (VEGF) receptor family are the RTK inhibitors being most widely studied. These agents target tumour cells or the tumour environment including stromal cells, endothelial cells, endothelial precursor cells, pericytes, and immune cells.

Tumor growth and progression depends very much on angiogenesis. VEGF is an endothelial cell-specific growth factor. It is indeed the principal regulator of angiogenesis in most organs, under normal as well as pathological conditions. It promotes the growth and the propensity to metastasize in several cancers, including endometrial cancer.

ErbB receptors and their ligands promote tumourigenesis by stimulating and accelerating tumour growth, survival, metastasis, invasion, angiogenesis and drug resistance.

HER-2*neu* expression has been found to be an independent prognostic factor in endometrial cancer. It is one of the critical oncogenes in high-grade and poor prognostic endometrial cancers. Inhibition of this receptor in poor prognostic endometrial cancer may be of some potential benefit.

The mitogen activated protein kinase (MAPK) pathway induces growth factors to promote survival and proliferation of tumor cells as well as normal cells.

Endometrial tumours show alterations in expression and activity of protein kinase C (PKC) isoforms, which seem to co-regulate apoptotic cell death.

CALAA-01 a small interfering RNA (siRNA), inhibits tumour growth by means of RNA interference thus reducing expression of R2, the M2 subunit of ribonucleotide reductase.

Uterine papillary serous cancers have been found to often overexpress claudin-3 and claudin-4 membrane proteins, which have been reported to be implicated in tumour invasion and tumor metastatic potential.

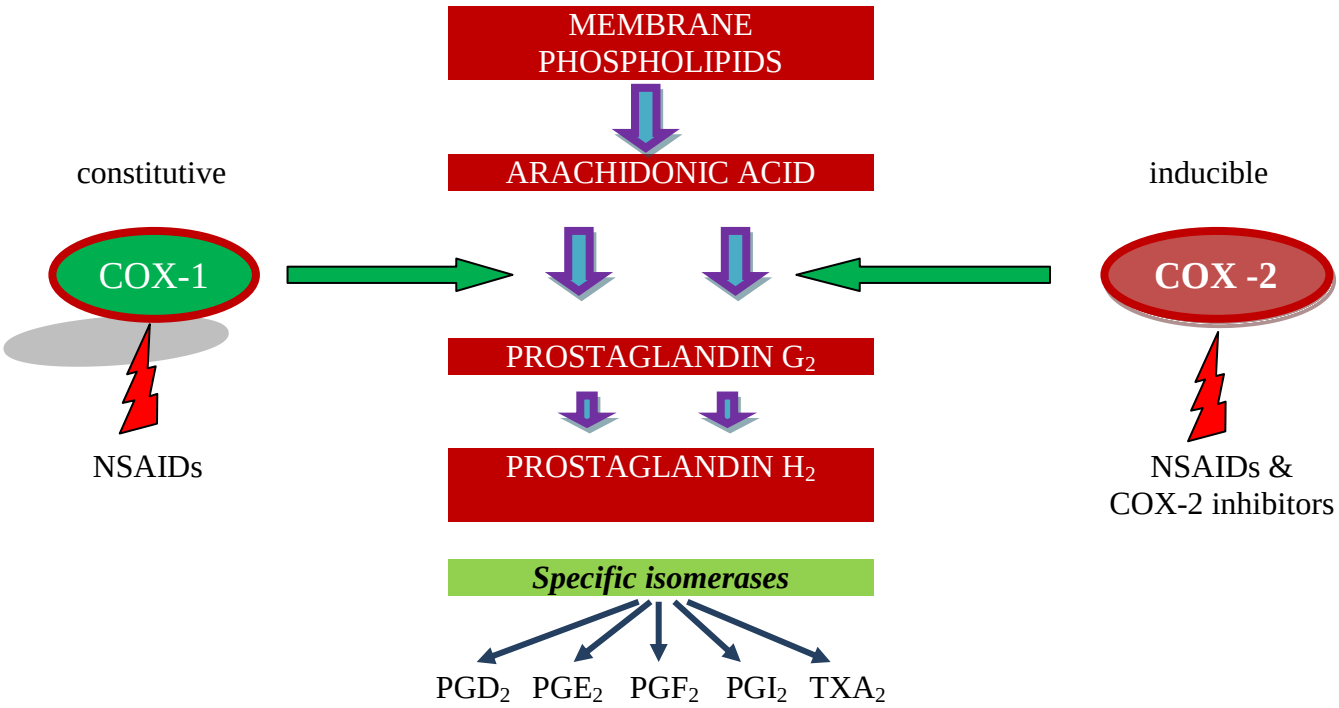
COX-2

BASIC INFORMATION ON COX-2:

Cyclooxygenase (COX) is an enzyme which catalyzes the conversion of arachidonic acid to prostaglandin G2. This is the rate limiting step in the synthesis of prostaglandins. Prostaglandin G2 is then converted to

prostaglandin H2 from which prostaglandins D2, E2, F2, I2 and thromboxane A2 are derived. There are two isoforms of cyclooxygenase – COX-1 and COX-2. The COX-1 isoform, is the enzyme involved in this process in normal conditions and it is involved in protection of gastric mucosal cells, platelet aggregation and renal vasodilatation. COX-2 is an inducible isoform that is expressed in the presence of cytokines in inflammatory conditions. It has also been found to be stimulated by oncogenes, tumor promoters and growth factors ***(Figure 1).***

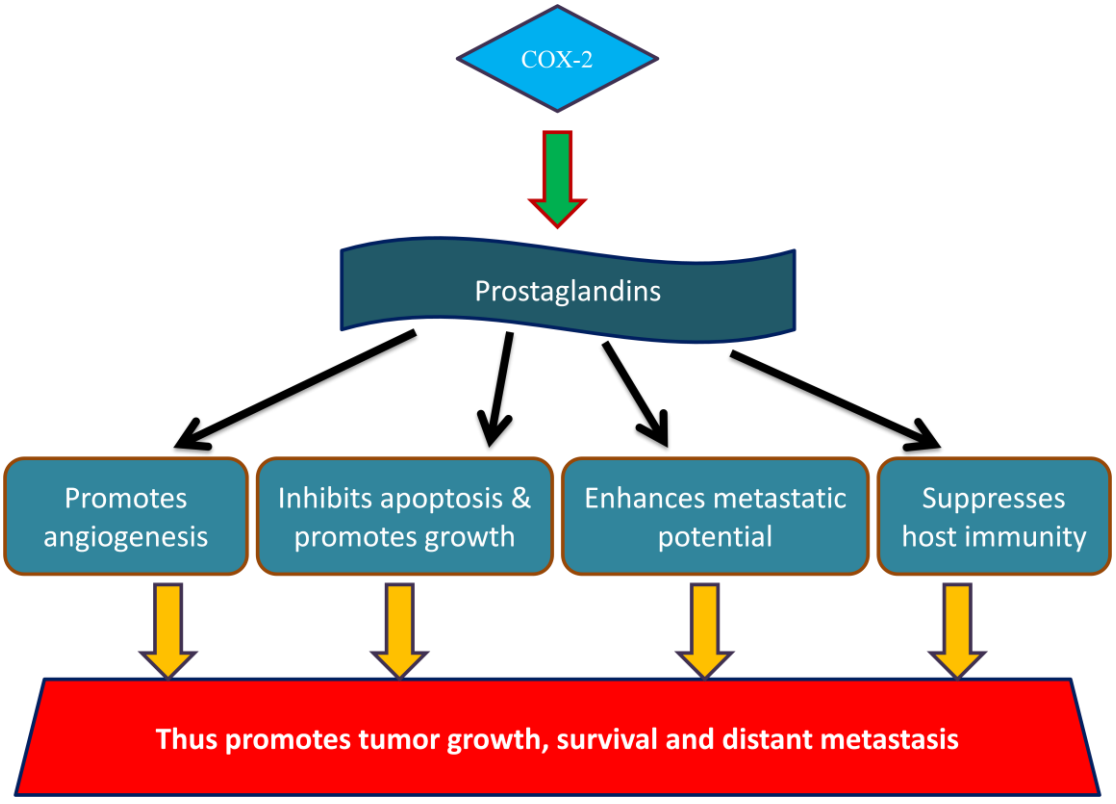
Figure 1: The arachidonic acid pathway



INVOLVEMENT OF COX-2 IN TUMORIGENESIS:

COX-2 has been shown to be constitutively expressed in various malignant tumors. It is considered to promote tumorigenesis by promoting angiogenesis, inhibiting apoptosis, inducing cell proliferation, enhancing the metastatic potential and by suppressing host immunity ***(Figure 2).***

Figure 2: COX-2 in tumorigenesis and tumor progression

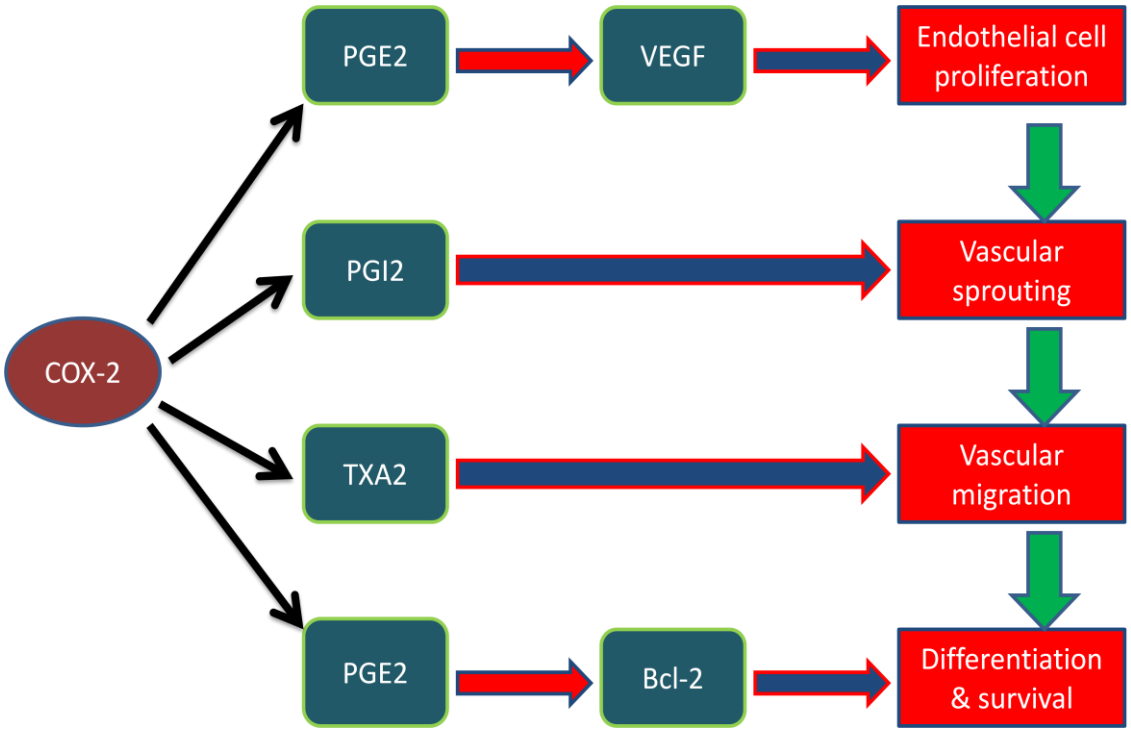


Angiogenesis:

Prostaglandin E2 induces the production of VEGF, matrix metalloproteinases and Bcl-2, all of which are involved in promoting endothelial cell proliferation. Prostacyclin induces the proliferating

endothelial cells to form new vascular sprouts. The endothelial cells produce proteases which facilitate vascular sprouting. Thromboxane A2 promotes the formation of capillary tubes by the migration of the proliferating endothelial cells towards the tumor site. Bcl-2 imparts survival advantage to the newly formed vessels ***(Figure 3).***

Figure 3: COX-2 in angiogenesis



Prevention of apoptosis:

COX-2 overexpression causes increased production of Bcl-2 which inhibits apoptosis. This increase in Bcl-2 production is due both to the reduction in the concentration of arachidonic acid and the increased production of prostaglandin E2 which activates the EP (Prostaglandin E2) receptors. The activation of EP receptors also leads to down-regulation of nitric oxide signaling which can further prevent apoptosis. The concentration of ceramide, an apoptosis inducer, within cells parallels that of COX-2.

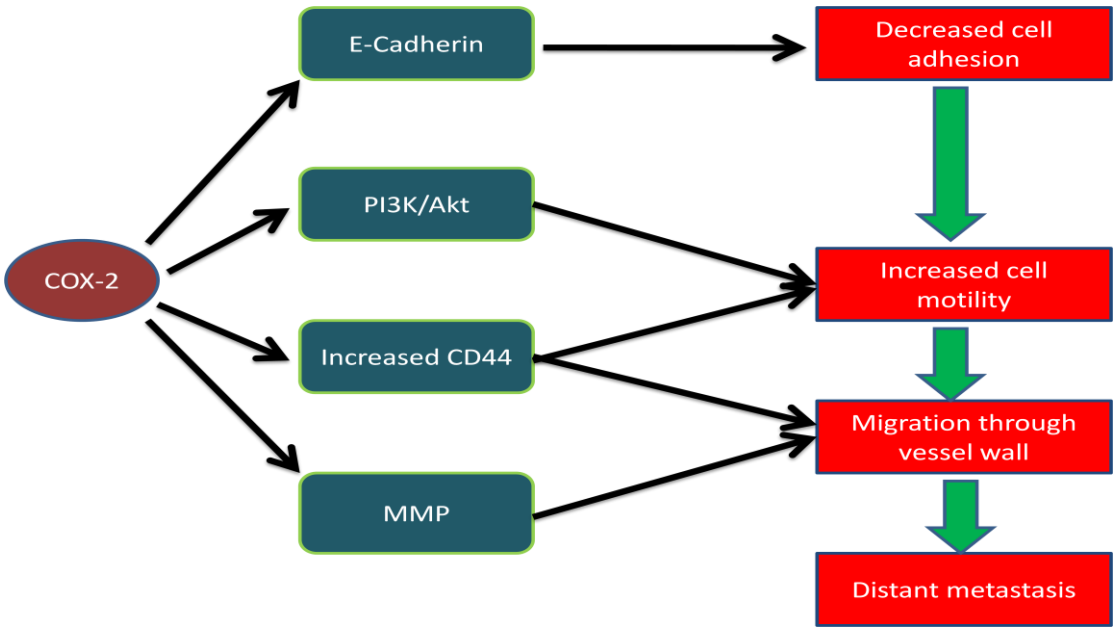
Induction of cellular proliferation:

PGE2 acts via the EP receptors which activate phosphatidylinositol 3 – kinase / protein kinase B pathway (PI3K/Akt) which promotes cellular proliferation.

Metastatic potential:

COX-2 decreases the adhesion of cells with one another by decreasing the expression of E-cadherin. These dyscohesive cells then acquire motility via the up-regulation of the PI3K/Akt pathway. The invasion of vessel wall by these migrating cells is facilitated by membrane metalloproteinases. Both cell migration as well as invasion is dependent upon the presence of the cell surface receptor CD44 [(40)] ***(Figure 4).***

Figure 4: COX-2 increases metastatic potential



Suppression of the host immune system:

Prostaglandin E2 inhibits the functions of antigen presenting dendritic cells. It also inhibits T cell activity by preventing the production of interleukin-2 (IL-2) and interferon-γ (INF–γ) as well as by decreasing the IL-2 receptor expression. The fact that COX-2 mediates immune suppression in tumor tissues has been validated by DeLong et al. who demonstrated the reinstatement of the anti-tumor response of the host immune system by the administration of COX-2 inhibitors [(41)].

EXPRESSION OF COX-2 IN VARIOUS TYPES OF NEOPLASMS:

The reduced incidence of colonic cancers in non-steroidal anti-inflammatory drug (NSAID) users has been the stimulus for scientists to study COX-2 expression in tumors [(42)].

Molecular studies performed at tissue level showed that, while COX-2 was either undetectable or expressed weakly in normal tissues harboring the cancer, it was overexpressed in cancer tissues. COX-2 is found to be overexpressed in many solid cancers such as head and neck tumors, esophagus, stomach, lung, breast, bladder, prostate and pancreas [(43)].

The first major study in India was published in 2011 by scientists from the department of Virology, King Institute of Preventive Medicine and Research, Chennai, where they observed overexpression of COX-2 in breast and lung cancers [(44)].

In the same year, a study from the CSM Medical University, Lucknow showed that COX-2 was overexpressed in about 16% of squamous cell carcinomas of oral cavity and it was more common in larger tumors than small sized tumors [(45)].

COX-2 overexpression is more common in invasive cervical squamous cell carcinoma and adenocarcinoma than in in-situ carcinoma [(46)]. Up regulation of COX-2 in bladder cancer and its dependence on TLR-4 was observed by Priyadarshini et al [(47)]. The most recent article published in 2014 by Soni

Kumari, Shashi Prakash Mishra, Rahul et al, showed that the incidence of COX-2 overexpression increased with the stage and with the presence of Helicobacter pylori in gastric cancer [48]. Thus, many centers in India have reported overexpression of COX-2 in various cancers and most of the studies have shown that the expression increases with tumor stage and size.

COX-2 IN ENDOMETRIAL CARCINOMAS:

Although COX-2 is either weakly expressed or not expressed in most of the normal tissues in the body, it has been found to be expressed by the normal glandular epithelium of the endometrium [40].

Studies of COX-2 expression in endometrial carcinomas started getting published in 2000 when Tong et al., found that COX-2 is overexpressed in endometrial carcinoma cells compared to normal endometrial cells [49].

COX-2 expression was found to be up regulated in endometrial cancer, being localized to the neoplastic epithelial cells as well as the normal endothelial cells, which were used as an inbuilt control in the study by Sugimoto et al [50].

Ferrandina et al in 2002 found a higher COX-2 positivity in endometrial carcinomas of stage II and above than in tumors presenting at stage I. There was also an increase in COX-2 positivity with worsening histological grades (*P* - 0.0049). Cases with deep myometrial invasion showed a significant correlation with COX-2 overexpression (*P*=0.0003). Also the P value

showed statistical significance for shorter disease free survival among the cases which overexpressed COX-2 (P= 0.028) [51].

Lambropoulou et al in 2005 have found that COX-2 overexpression correlated significantly with the FIGO stage of the endometrial carcinomas (p=0.010). A positive correlation was also found with histological grade (p=0.019) and myometrial invasion (p=0.026). However, COX-2 overexpression did not correlate with the histologic type of the tumor (p=0.164). There was however a significant correlation between COX-2 overexpression and reduced survival rates (p=0.028). They thus suggest that COX-2 overexpression is an independent clinicopathological and prognostic factor in endometrial cancers [52].

Ferrandina et al in 2005 and Orejuela et al in 2005 did not find any correlation between COX-2 positivity and any of the clinicopathological features [53, 7].

Horn et al studied the expression of COX-2 in the more aggressive squamous cell carcinomas of the endometrium and found it to be overexpressed in all eight of their cases [8].

Fowler et al in 2005 found that COX-2 expression correlated well with histologic grade (P < .026) and approached statistical significance for deep myometrial invasion (P < .055) [54].

Li et al in 2006 found the overexpression of COX-2 in 64.7% of endometrial cancers but not in normal endometrium. They found that grade 2 tumors overexpressed COX-2 at a higher frequency than grade 3 tumors at $P < 0.05$). They have therefore suggested that overexpression of COX-2 may be involved in induction of VEGF expression, upregulation of angiogenesis, and enhancement of tumor growth [(55)].

Nasir et al in 2007 have found a high COX-2 positivity rate in endometrial adenocarcinoma tissues (88%) as compared to endometrial hyperplasias and non neoplastic endometrium [(6)]. These observations are

similar to those reported in other studies as well [(56)], while some investigators have found comparable proportions of endometrial carcinomas (92%) and proliferative endometrium (86%) to express COX-2 [(57)].

Keser et al in 2010 did not find a statistically significant difference between subgroups of histological grade, myometrial invasion and COX–2 scores. In addition, they were not able to establish a statistically significant correlation between steroid receptor status and COX-2 expression at $P < 0.05$ [(58)].

In another study by Cao JQ and team, COX–2 was not expressed by atrophic, proliferative and hyperplastic endometrium. There was a weak to moderate granular cytoplasmic expression in well-differentiated endometrial carcinoma. There was however a strong expression of COX-2 (overexpression) in the more poorly differentiated tumors. According to the

authors, the overexpression of COX–2 in moderate to high grade endometrial carcinomas suggested that COX–2 was involved in tumor progression rather than tumor initiation [59].

Erkanli et al. could not demonstrate any association between overexpression of COX–2 and prognostic factors like myometrial invasion, grade or stage in endometrial cancers [60].

Jeon et al. reported that COX–2 immunoreactivity of normal endometrial glands and stroma was minimal, irrespective of menstrual phase. In contrast, tumor cells showed significant immunoreactivity for COX–2.

Twenty-seven of the 152 specimens studied were COX–2 positive (17.8%). COX–2 overexpression was not associated with a statistically significant correlation with any of the clinicopathological parameters studied by the authors except for the menstrual status. They observed that post menopausal women overexpressed COX-2 more frequently than premenopausal women [61].

In a study by Ohno et al. which consisted of 70 endometrial carcinoma cases, the expression of COX–2 was strong in 37 (53%) patients and weak in 33 (47%) patients. They found increasing COX-2 overexpression with worsening tumor stage and deep myometrial invasion but were not able to establish a statistically significant association [62].

In 2005, Hasegawa et al studied the effects of the selective COX-2 inhibitor, Etodolac on endometrial carcinoma cell line called TMG-L, which was found to overexpress COX-2. They demonstrated that Etodolac caused inhibition of cell proliferation in this cell line by arresting the cell cycle at G_1 phase. The effect of the drug was found to be dose dependent. They hypothesized that this cell cycle arrest is brought about by an increase in the expression of p53 and P21WAF1 proteins caused by COX-2. The drug was also found to inhibit the production of basic-Fibroblast Growth Factor (b-FGF) and the activity of the telomerase enzyme [(63)]. Later in 2012, they followed up this study with another in which a group of 21 patients with endometrial carcinoma were administered Etodolac prior to surgery. COX-2 overexpression was present in 16 and absent in 5 cases. They obtained the endometrial cytology samples after the treatment trial and studied the cellular features. They found a reduction in the mitotic index in all of the cases which had overexpressed COX-2. There was also a reduction in the nuclear atypia, anisokaryosis, hyperchromasia and nuclear/cytoplasmic ratio in some of these cases. Further, they did not find any alterations in the cytologic features, in cases that did not over express COX-2. They therefore concluded that, Etodolac, a selective COX-2 inhibitor, has antitumor effects on endometrial cancer tissue [(64)].

Scoring systems for COX-2 immunostaining:

Literature that evolved on the detection of COX-2 in endometrial tissues and cancers showed that the expression was higher in cancers. This necessitated the need to define overexpression to differentiate from mere expression. Several scoring systems are in vogue whose common denominator is bringing out objectivity in reporting and to eliminate bias. The following is a review of the scoring systems used to define overexpression of COX-2.

1. Fujiwaki, et al, in 2001, gave a score of 0-4 for the percentage of cells stained: 0 for none, 1 for 1-25%, 2 for 26-50%, 3 for 51-75%
   and 4 for 76-100% and a score of 0-3 for the staining intensity: 0 for none, 1 for week, 2 for moderate and 3 for intense. The intensity was compared with the staining intensity in smooth muscle cells which was given an arbitrary score of 2. As the staining intensities varied in different parts of the same tumor, the score of the intensity and percentage of cells stained, in the various parts of the tumor were multiplied and the products were summed up to give the final score [(56)].
2. Jeon, et al, in 2004, used a simpler system. They used a median value of 5% as the cut off for defining COX-2 overexpression by the tumor cells [(61)]. However, Ferrandina et al raised the median value to 26% in their study [(53)].

3. Fowler, et al, in 2005, gave a combined score for the staining intensity and the percentage of cells stained. A score of 3+ was given for strong cytoplasmic staining in >50% cells, 2+ for weak cytoplasmic staining in >50% cells, 1+ for strong or weak cytoplasmic staining in 50% - 10% of cells and 0 for staining of <10% of cell or no staining. A score of 2+ or 3+ was considered as overexpression [(54)].
4. Orejuela et al, used only the percentage of cells stained for scoring the slides. Their scores were: 0 for no staining, 1 for staining in <10% of cells, 2 for staining in 10-50% of cells and 3 for staining in >50% of cells. Scores 2 and 3 were considered as overexpression [(7)].
5. Li, et al, in 2006, used a similar system for scoring as Fujiwaki, et al, but instead of multiplying the scores for the staining intensity and the percentage of cells stained they summed up the scores and considered a final score of 3 or above to be indicative of COX-2 overexpression [(55)].
6. Erkanli, et al, in 2007, followed the same scoring system as Li, et al, but the cut off for overexpression was increased to a final score of 4 or more [(60)].

7. Nasir, et al, in 2007, used a semi quantitative scoring system in which an intensity score of 0-3 (0- negative, 1+ - weak, 2+ - medium and 3+ - strong) was given and the percentage of cells that have taken up the stain were identified and the final score was arrived at using the formula- (3 x % of cells staining 3+) + (2 x % of cells staining 2+) + (1 x % of cells staining 1+). They used the vascular endothelial cells and macrophages as the positive internal control [(6)].

Type of study:

This is a retrospective study performed on all consecutive cases of endometrial carcinoma, reported on hysterectomy specimens from the year 2007 to 2013, in the Department of Pathology of the PSG Institute of Medical Sciences and Research.

Arriving at the study population:

The histopathology registers of the years 2007 to 2013, maintained in the Department of Pathology were accessed to obtain the following set of information:

1. The total number of biopsies received in the Department of Pathology of PSG Institute of Medical Sciences and Research during the study period.
2. The number of biopsies that were reported as malignant.
3. Among the malignancies, the total number of endometrial carcinomas reported.
4. Of the total number of endometrial carcinomas, the list of cases for which the diagnosis was made on a resection specimen. This list was the study population.

Retrieving the study material:

All the histopathology slides of the study population were retrieved. The slides of all these cases were reviewed, first by me and then by my guide. We identified the most representative slides containing a fair amount of the tumor material. As a thumb rule, we chose those slides, which had a minimum, well-preserved tumor area of 40% – 50% with necrotic tissue not exceeding 20% of the total tissue. This was done to prevent staining artifacts while performing immunohistochemical studies. The specific histopathology numbers of these slides were noted and the corresponding blocks were retrieved from the archives with the help of the histopathology support staff.

Two slides were prepared from each of these blocks, one of which was stained with the hematoxylin and eosin stain and the other with the COX-2 immunostain.

Procedure of Hematoxylin and Eosin staining:

This comprised four sequential processes. These were section cutting of the chosen paraffin blocks, deparaffinization, staining proper and mounting. Section cutting was performed on Leica semi-automated

microtome fitted with disposable cutting blades. Deparaffinization and staining was done in a fully automated robotic auto-stainer (Leica Autostainer XL) using a program, specific to H&E staining. Mounting was done manually.

As a first step, 4-5 micron thin sections were cut and floated onto two slides. One of the slides was a frosted slide coated with egg albumin. This slide was for Hematoxylin and Eosin staining. The other slide was a frosted slide coated with poly-L-lysine for immunohistochemical study. The sections floated onto egg albumin coated frosted slides were air dried after which the slides were placed in the Leica Autostainer for deparaffinization and staining. The program schedule for these activities was as shown below:

i. Heating in an oven for 6 minutes at 65^{o}C to liquefy paraffin wax.
ii. Two changes in 97% Xylene for 5 minutes each to remove molten wax.
iii. Transfer of slides to a 1:1 mixture of xylene and alcohol for 30 seconds.
iv. Three changes in 99% alcohol for 30 seconds each, to remove Xylene.

v. One change in tap water for 30 seconds, to wash off excess alcohol.

vi. Harry's hematoxylin for 5 minutes, for nuclear staining.

vii. One change in tap water for 1 minute, for bluing of the hematoxylin.

viii. Removing the excess stain by differentiating in 1% acid alcohol for a period of 30 seconds, followed by a wash in tap water for 2 minutes (regressive staining).

ix. Staining with 1% eosin for 2 minutes – cytoplasmic staining.

x. Washing off the excess stain in tap water for 30 seconds.

xi. The slides were then air-dried, cleared with Xylene and finally mounted using Distyrene Phthalate Xylene (DPX).

Procedure of Immunohistochemistry (IHC)

Immunohistochemistry staining was performed manually. The steps were:

1. 4 to 5μm thickness sections were cut and floated on to poly- L- lysine coated slides.

2. Incubation at 37^{o}C for 12 hours during the day and then at 58^{o}C for the next 12 hours.

3. Deparaffinization was performed using two successive jars of xylene for 15 minutes each.

4. The xylene was then removed in two successive changes of absolute alcohol for 1 minute each.

5. The sections were then treated for one minute each in 90% alcohol and 70% alcohol.

6. Rehydration was carried out using tap water for 10 minutes.

7. The slides were then rinsed in distilled water for 5 minutes.

8. Antigen retrieval was done by pressure-cooking the sections in citrate buffer at a pH of 6.0 for 10 minutes.

9. The cooker was cooled to room temperature by placing it in a sink filled with water for 20 minutes.

10. The slides were rinsed in distilled water for 5 minutes.

11. They were then washed twice in phosphate buffered saline at a pH of 7.6 for 5 minutes each.

12. Endogenous peroxidases which may compete with the reagent horseradish peroxidase were blocked using hydrogen peroxide for 10-15 minutes.

13. The slides were washed in phosphate buffered saline for 5 minutes.

14. Non specific reaction of the reagent antibody with other tissue antigens was blocked using Power Block, which is a universal protein block containing casein and sodium azide.

15. The sections were then covered with the reagent COX-2 antibody and left undisturbed for an hour.

16. Any unbound antibody present was washed off using phosphate buffered saline for 3 minutes.

17. To enhance the signals produced, the sections were covered with Super Enhancer for 3 minutes and the slides were washed thrice in phosphate buffered saline for 5 minutes each.

18. The sections were then covered with Super Sensitive Polymer HRP, an anti-species immunoglobulin labeled with an enzyme polymer, for 30 minutes.

19. The unbound antibody was removed by washing thrice in phosphate buffered saline.

20. The chromogen DAB 3,3'- diaminobenzidine, was added on to the sections and left for 5-8 minutes, during which time a brown precipitate was formed.

21. The slides were washed thrice in phosphate buffered saline and then in tap water.

22. Nuclear counterstaining was done using Mayer's hematoxylin for 1 minute.

23. The slides were then washed in tap water, air dried, cleared using xylene and mounted with DPX.

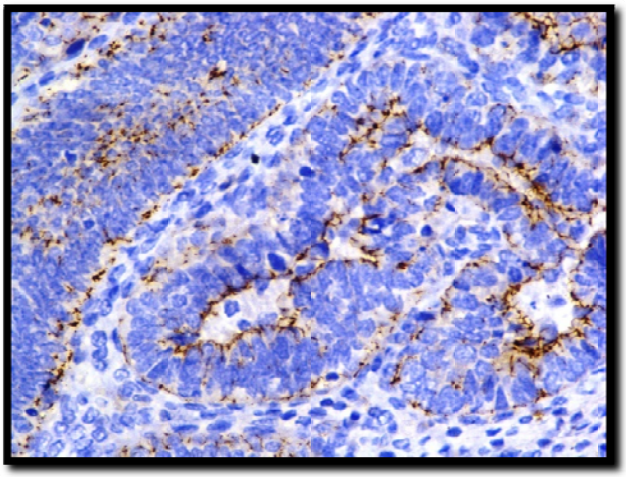
We used COX-2 as the primary antibody for the above procedure without involving any changes in any of the steps performed. We used a rabbit monoclonal IgG antibody (clone SP21), of Thermo Fisher Scientific make. It was in a ready to use form bearing a catalogue number RM-9121-R7 (prediluted in 0.05mol/L Tris-HCL, pH 7.6 with added stabilizing protein and 0.015mol/L sodium azide). The lot number was 9121R1209A and its expiry date was 21.09.2014. We used a representative block from a case of colorectal cancer diagnosed in our institute as a positive control, as recommended by the data sheet provided by the manufacturers.

Scoring of slides:

The presence of representative tumor tissue in the archival blocks was confirmed once again by reviewing the freshly cut hematoxylin and eosin stained slides obtained from these blocks. The IHC slides were also

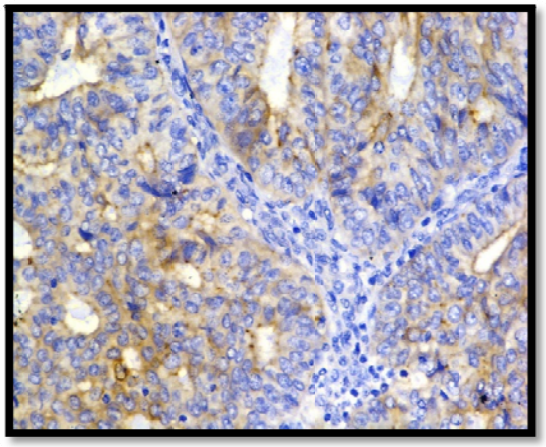
reviewed to look for the presence of tumor material. Once it was confirmed that the sections had adequate tumor tissue, scoring of the staining was performed to classify the results into negative, positive and overexpressing. We used the two tier scoring system described by Serkan Erkanli, et al [(60)]. Firstly, the staining intensity was given a score ranging from 0 to 3 (0 for negative, 1 for weak, 2 for medium and 3 for strong). Secondly, the percentage of cells that have stained was scored. It ranged from 0 to 4 (0 for 0%, 1 for 1 to 25%, 2 for 26 to 50%, 3 for 51-75% and 4 for 76-100%). The overall score was the sum of the two scores, which ranged from 0 to 7. Cells were defined as positive for COX-2 if the staining was of cytoplasmic type. Staining of nucleus, nuclear membrane or cell membrane alone was considered negative ***(Figure 5).***

Figure 5: Negative immunostaining for COX-2

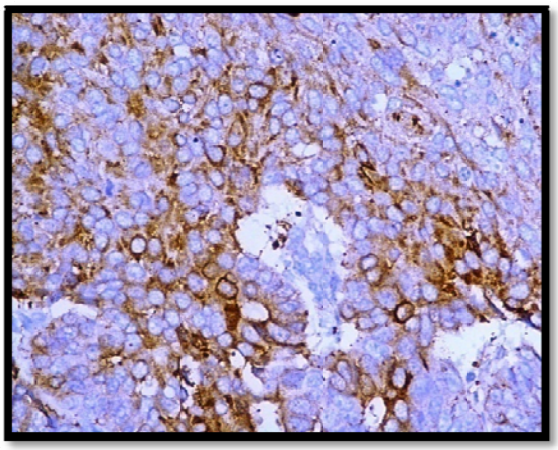


Staining for COX-2 immunomarker shows absence of staining of the cytoplasm. Staining of cell membrane alone, as seen in this picture, is considered 'negative'.

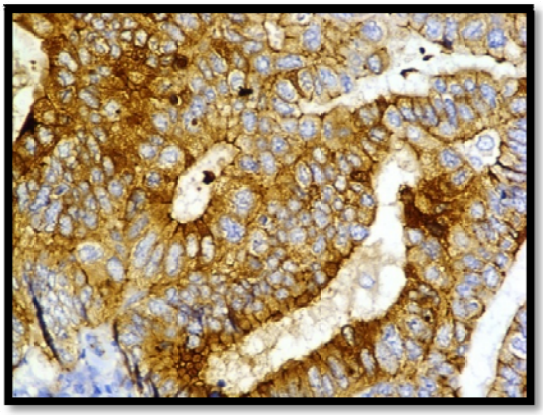
Figure 6: Positive immunostaining for COX-2



The presence of faint and diffuse cytoplasmic staining was scored as 'weak' (score = 1).



The presence of moderate to strong granular cytoplasmic staining was scored as 'moderate' (score = 2).



The presence of strong and diffuse cytoplasmic staining was scored as 'strong' (score = 3).

To reduce subjectivity in scoring the intensity of staining, we adopted the method described by Robert Sitarz, Roos J Leguit, Wendy WJ de Leng et al[65] ***(Figure 6).***

Accordingly, we categorized faint and diffuse cytoplasmic staining as 'weak'(score = 1), moderate to strong granular cytoplasmic staining as 'moderate' (score = 2) and strong and diffuse cytoplasmic staining as 'strong' (score = 3) [65].

We used tissues of an old case of colon cancer that was strongly positive for COX-2 as a positive control.

I did the scoring first and my guide reviewed the results.

Based on the overall scores, the tumors were classified as negative for COX-2 immunostaining if the total score was 0 and positive if the scores ranged from 2-7. Of the positive cases, COX-2 was considered to be overexpressed if the total score was $\geq$4.

Data entry:

A master-chart was prepared. For each of the study cases, which were identified by a unique histopathology ID number, the following parameters were noted:

i. Age of the patient
ii. The parity index
iii. The menstrual status
iv. The clinical stage
v. The histological type of the tumor
vi. The tumor grade, and
vii. The extent of myometrial invasion

Information on the first four parameters was obtained from the patients' case records maintained in the Medical Records Department of the institution. Information on the remainder three parameters was from the histopathology records maintained by our department.

We created additional columns to add the scores of intensity of IHC staining, percentage of cells that stained positive and the overall score to identify overexpression. Cases with a negative score, as well as those, which did not overexpress COX-2, were clubbed together as negative for overexpression and those cases, which had a score of four or more, were designated as positive for overexpression, for the purpose of statistical analysis.

Data analysis:

The number of cases overexpressing COX-2 was expressed as a percentage of the total number of endometrial cancers. The COX-2 expression pattern was then analyzed against each of the above mentioned variables. Chi-square test was used wherever it was found necessary to assess the level of significance. We assigned a ‘p’ value of <0.05 as statistically significant.

The study period was from 1st January, 2007 to 31st December 2013. During this period, the Histopathology division of the Department of Pathology received a total of 35,449 specimens. Thus the average biopsy load per year (based on the statics of the study period) was 5064.

During this period, 3406 biopsies were reported as malignant (i.e) the occurrence of malignancies per year is 9.6%. Endometrial carcinomas accounted for 1.6% of the total malignancies (54 out of 3406).

Of the 54 endometrial malignancies 39 cases were diagnosed on a resection specimen. For two of the cases, the blocks were not available. Hence, the study population is 37 cases of endometrial carcinoma that were diagnosed on resection specimens, for which blocks were available for immunohistochemical studies with COX-2 immunomarker.

An analysis of the study population showed that, the mean age at diagnosis was 57.5 years and the median age was 59. Majority of the patients (70%) were in the 6th and 7th decades of life ***(Figure 7)***. 68% of endometrial cancers occurred in women who have had three or less of pregnancies ***(Figure 8)***.

Figure 7: Age distribution of endometrial carcinoma

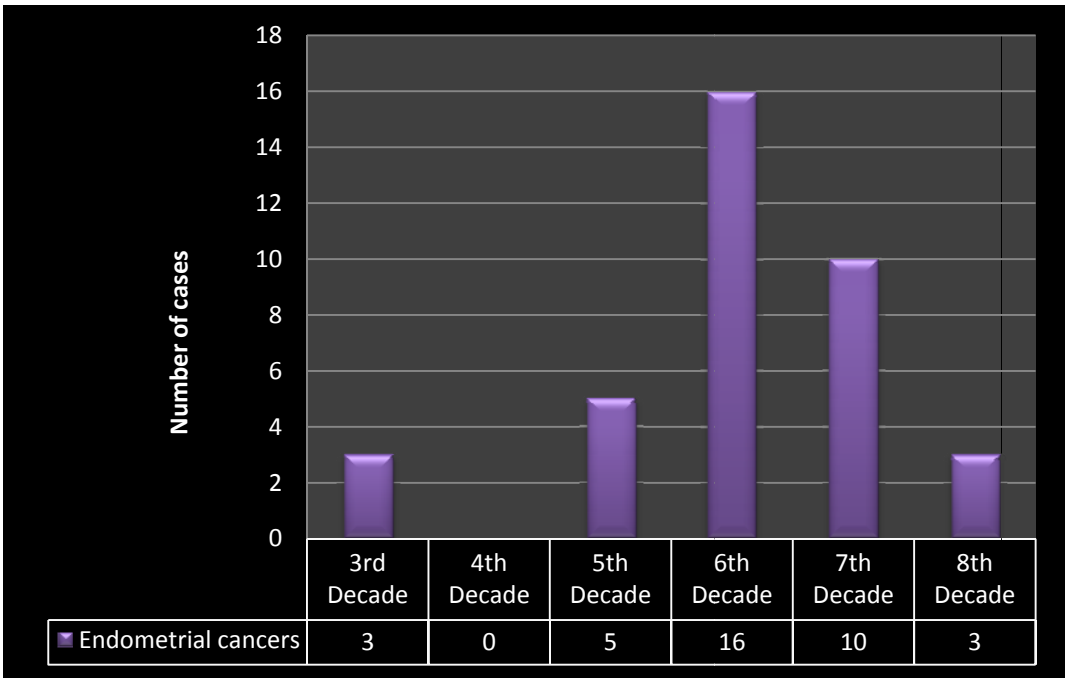
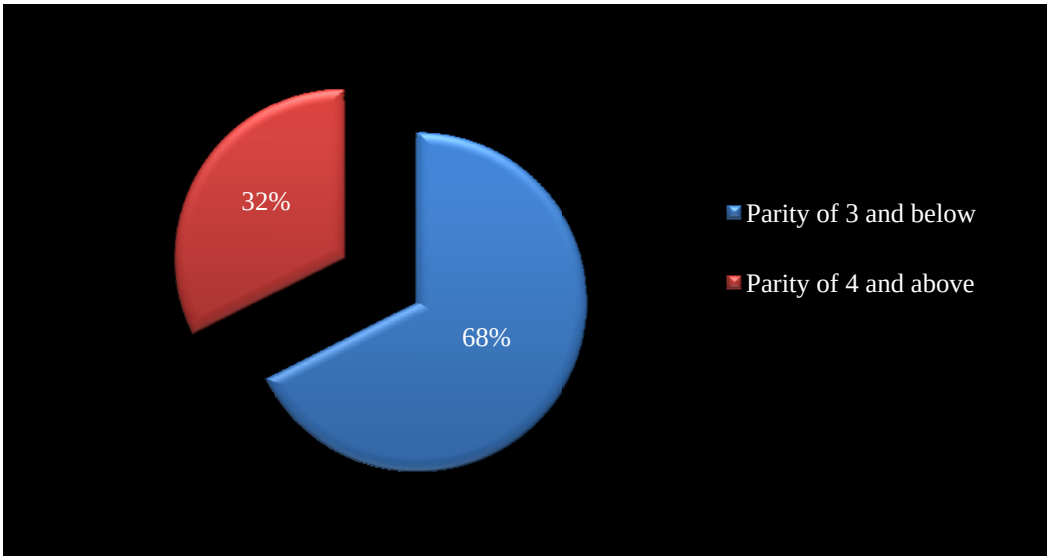


Figure 8: Endometrial carcinoma and parity



The most common histological type of endometrial carcinoma in our study was the endometrioid type, which constituted 89% of cases (33 out of 37). Four cases of endometrial carcinomas were of the non-endometrioid type, one each of mucinous, squamous, serous and clear cell type ***(Figure 9)***.

59.5% (22 out of 37) of endometrial cancers have been reported to be of grade I (FIGO grading system), making it the commonest grade at presentation for endometrial carcinoma in our study ***(Figure 10)***. Only 3 cases (8%) were of grade 3.

Figure 9: Distribution of histologic types of endometrial carcinoma

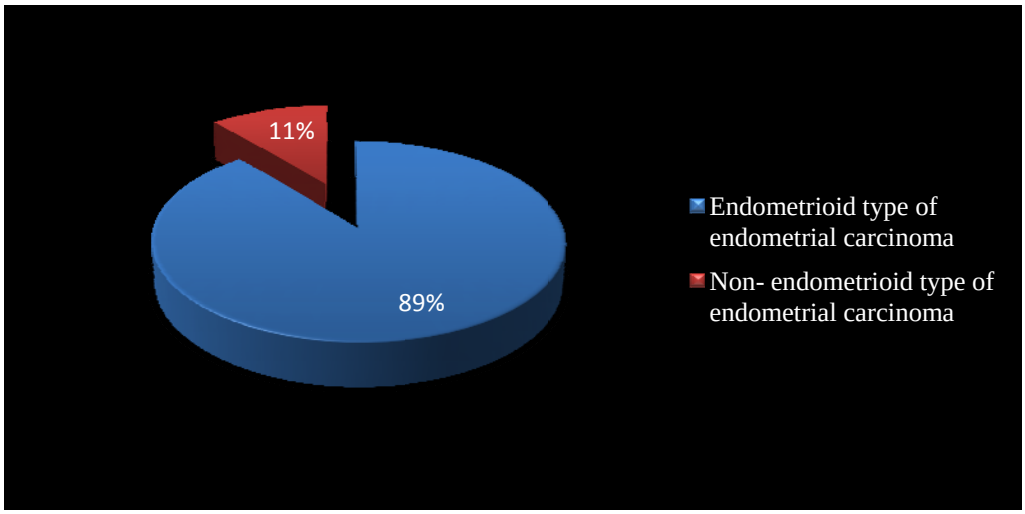
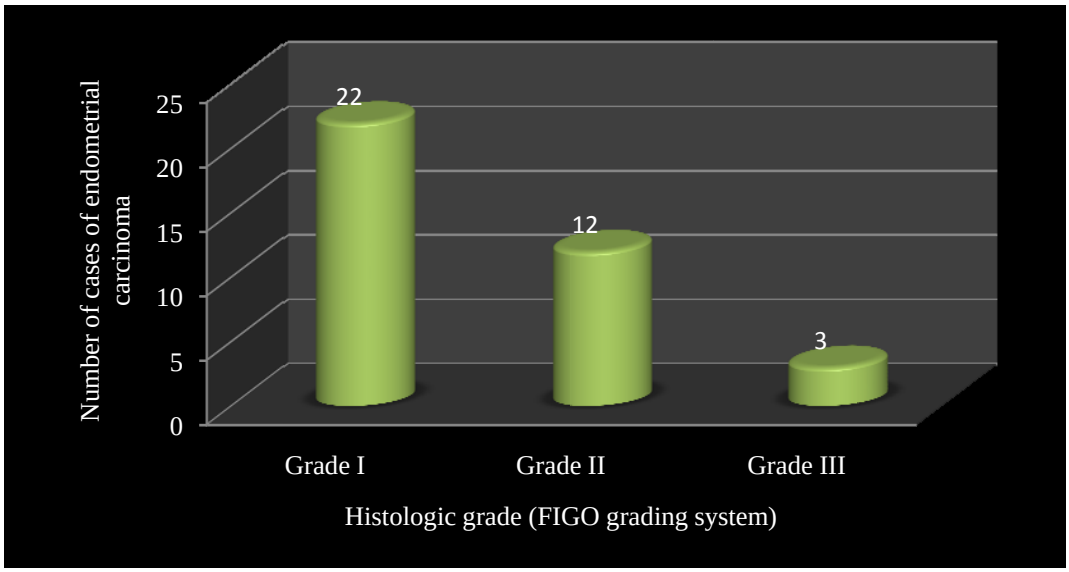
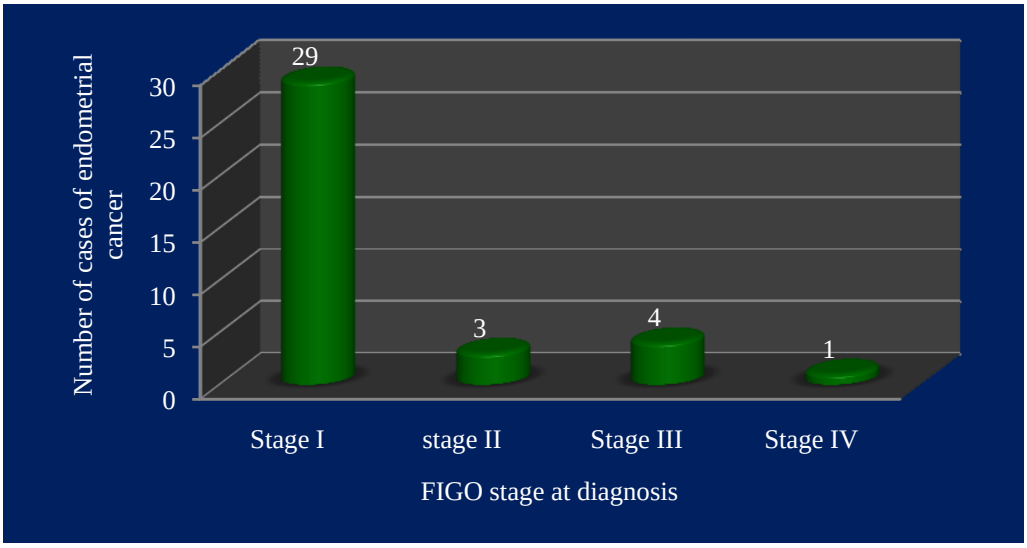


Figure 10: Distribution of histologic grades of endometrial carcinoma



FIGO staging was reported on all the cases of endometrial carcinomas. 29 out of 37 cases were diagnosed to have stage I disease (78%). One of the cases presented with a stage IV disease on diagnosis ***(Figure 11)***.

Figure 11: FIGO stage of endometrial carcinoma



Immunostaining for COX-2 was performed on all the 37 cases. All but one of them showed expression of the immunomarker (97% expression). The mean score of the percentage of cells that were positive for the immunomarker was 3.3 (of a maximum of 4), while the mean intensity score was 1.7 of a maximum of 3. The mean overall score was 5 of a maximum of 7.

Cases with an overall score of 4 and above were considered to be positive for overexpression of the COX-2 immunomarker, while those that had an overall score of 3 and below were considered negative for overexpression. Thus 78% (29 out of 37 cases) of the endometrial cancers were found to be overexpressive for the COX-2 immunomarker.

As seen in ***table 4,*** 29 of the endometrial cancers were in stage I of which 21 showed overexpression (73%). The remainders 8 were in stage II and above. All of them showed overexpresion (100%). The p value for COX-2 overexpression between patients in stage I and those in stages II, III & IV is not significant (0.093).

17 out of 22 cases (77%), whose histologic grade was FIGO grade I, were positive for overexpression of COX-2 ***(table 5).*** Similarly, 75% of the cases (9 out of 12) whose histologic grade was FIGO grade 2 were found

to be overexpressive. 100% overexpression was found in all the 3 cases whose histologic grade was FIGO grade 3. The p value for COX-2 overexpression between patients in the three different histological grades was also not significant (0.63).

Table 4: COX-2 overexpression and FIGO stage

FIGO Stage	Positive for overexpression for COX-2	Negative for overexpression for COX-2	Total
I	21	8	29
II	3	0	3
III	4	0	4
IV	1	0	1
Total	29	8	37

All the 4 non endometrioid endometrial carcinomas were positive for overexpression of COX-2 ***(table 6).*** Of the 33 endometrioid endometrial carcinomas, 24% (8 cases) were positive but not overexpressive for COX-2. The p value for COX-2 overexpression between patients belonging to endometrioid and non-endometrioid groups was not significant (0.27).

Table 5: COX-2 overexpression and FIGO grade

FIGO grade	Positive for overexpression for COX-2	Negative for overexpression for COX-2	Total
I	17	5	22
II	9	3	12
III	3	0	3
Total	29	8	37

Table 6: COX-2 overexpression and histologic type

Type	Positive for overexpression for COX-2	Negative for overexpression for COX-2	Total
Endometrioid	25	8	33
Non-endometrioid	4	0	4
Total	29	8	37

Table 7, shows that in 7 out of 9 (78%) of the cases, where the tumor has invaded more than half of the myometrial thickness, there was overexpression for COX-2. 79% of the remainder (cases where the tumor invasion was less than half of the myometrial thickness) also showed

overexpression for COX-2. The p value for COX-2 overexpression in patients with less or more than half of myometrial invasion is not significant (0.96).

Table 7: COX-2 overexpression and myometrial invasion

Myometrial invasion	Positive for overexpression for COX-2	Negative for overexpression for COX-2	Total
<1/2	22	6	28
>1/2	7	2	9
Total	29	8	37

Table 8: COX-2 overexpression and menopausal status

Menstrual status	Positive for overexpression for COX-2	Negative for overexpression for COX-2	Total
Premenopausal	4	1	5
Postmenopausal	25	7	32
Total	29	8	37

As seen in ***table 8,*** 4 out of 5 (80%) of endometrial carcinoma, that occurred in premenopausal women were found to be positive for overexpression of COX-2. 25 out of the 32 cases (78%), that occurred in postmenopausal women were positive for overexpression of COX-2. The p value for COX-2 overexpression in patients based on menopausal status is not significant (0.92).

20 out of 25 cases of endometrial carcinoma (80%) that occurred in women whose parity (gravida status) was 3 or below, overexpressed COX-2 ***(table 9)***. 75% of cases that occurred in women whose parity was greater than 3, were also positive for overexpression of COX-2. The p value for COX-2 overexpression in patients based on parity is not significant (0.73).

Table 9: COX-2 overexpression and parity status

Parity	Positive for overexpression for COX-2	Negative for overexpression for COX-2	Total
</= 3	20	5	25
>3	9	3	12
Total	29	8	37

PSG hospitals is a tertiary care teaching hospital affiliated to PSG Institute of Medical Sciences & Research, Coimbatore. The hospital is accredited by National Accreditation Board for Hospitals. All the diagnostic service laboratories, including histopathology and cytopathology are accredited by the National Accreditation Board for Laboratories.

The hospital receives patients from the city of Coimbatore and from the nearby districts. It is also the higher referral hospital for its patients referred from its rural outreach centers.

The histopathology division of the Department of Pathology receives specimens predominantly from patients operated within the premises of PSG hospitals. This is a very conducive situation for the reporting pathologists as the entire detail of the patients can be accessed online by a secure login into the Hospital Information System. The histopathology laboratory is categorized as a medium sized laboratory as it receives about 20 to 40 biopsy samples per day.

All the samples are received in 10% buffered formalin in wide mouthed containers. These are usually well labeled. A test request form, filled in by a member of the operating team, accompanies all of the specimens. The samples thus received in the laboratory are immediately indexed in the accession register and a unique laboratory number is generated which is transcribed on to the request form and the container. After grossing, the tissues for study are processed in an automated tissue processor after which the paraffin blocks are prepared at an automated embedding station. The paraffin blocks are cut into 4-5 microns thin sections using an automated rotary microtome. The entire staining is also automated. Thus, the laboratory ensures consistency in its activities, which is crucial for an accurate reporting. The senior-most trained and experienced technician performs immunohistochemistry manually. With every batch of immunohistochemistry study, positive and negative control sections are also provided to guide the reporting.

During the study period, 9.6% of the biopsy samples were reported as malignant. This figure may not accurately represent the occurrence of cancer in a tertiary care hospital because a few patients are lost to follow up after a clinical / radiological diagnosis of cancer. Further, some of the

cancers could have been reported twice- for instance, an excision biopsy following an initial trucut / wedge biopsy.

Since 2012, the institution has developed a data base to report all cancers to the National Registry for Cancer. This necessitated the need for incorporating the ICD-O codes for all cases reported as malignant. This system helps to prevent over reporting of cancer incidence as patients with similar data are immediately identified. Hence, the institution will be in a better position to report the exact occurrence of cancer in the years to come.

We observed that endometrial cancers accounted for 1.6% of the total cancers. This is almost comparable with the incidence reported by GLOBOCAN for endometrial carcinoma in India (2.3%), the incidence being adjusted for both the sexes. Some of the reasons for the lesser incidence in India compared to global figures could be early parity and multiparity. The incidence could go up as India is fast becoming the diabetic capital of the world, whereby women are more likely to experience excessive exposure to estrogens produced from peripheral fat reserves.

Our institution offers full time medical oncology services. These are in vogue since 2012. All those patients who had malignancies and required post operative radiotherapy usually visit one of the two radiation oncology centers in the city. These patients would carry with them either the slides or blocks to that hospital, either for confirmation of diagnosis or to perform additional molecular studies. Thus, of the 39 endometrial malignancies reported during the study period, blocks of two patients were transferred out. Hence, these cases had to be excluded.

The median age at diagnosis of endometrial cancer in the study population is very similar to that reported in literature. However, 8% of our patients were below the age of 40 years. This is much higher than the observations made by Robboy[(2)]. However, we admit that our data is too small to be compared with large studies published in literature.

Parity is considered an important risk factor for endometrial cancer as it provides estrogen free spells to the endometrium. India is experiencing an increasing trend towards nulliparity. This is because of more women opting to remain single and also due to increasing incidence of infertility. In our

study, only one of the patients with endometrial cancer is a nulliparous woman. This incidence could go higher in the future. The incidence could also rise as discussed earlier, due to an increasing population of obese and diabetic people. In our study, due to the very low incidence of nulliparity, we grouped the study population into two. The first group comprised of women who had conceived three or less number of times. Women with more than three conceptions were categorized as the second group. We did not take into account the actual parity, the number of abortions and the number of live children. We presumed that, conception as an event, provided a minimum three months estrogen free period to the endometrium. We observed that the majority of endometrial cancers occurred in the first group, thus implying the safety to the endometrium provided by a multiparous situation.

The occurrence of endometrioid type of adenocarcinoma of the uterus is similar to the observations in the literature [(28)]. Non-endometrioid type cancers were 11% of all endometrial cancers and comprised one each of mucinous, squamous, serous and clear cell types. Thus, there was no preponderance to a specific type. Fowler JM, Ramirez N, Cohn D et al found 18% of the endometrial cancers to be of the non-endometrioid type,

while Lambropoulou M, Alexiadis G, Limberis V et al observed that such neoplasms accounted for 14% of their study population [54,52]. Ferrandina G, Legge F, Ranelletti F et al l however, have reported that only 7% of their study population was of the non-endometrioid type and all of them were of the serous type [51].

About 60% of the endometrial cancers are of grade I histology [28]. In our study, we have graded both endometrioid and non-endometrioid cancers. For endometrioid cancers, an architectural grade was first arrived at. This was followed by an assessment of the nuclear grade. We did not observe any of the endometrioid carcinomas to display grade III nuclear morphology; hence, there was no requirement to increase the overall histologic grade. The non-endometrioid cancers were graded based on the nuclear features only. All of the grade III endometrial cancers were of the non endometrioid type. The lone non-endometrioid cancer presenting with a lesser grade was of the squamous type (Grade II).

FIGO stage I continues to be the most common stage at presentation for patients with endometrial cancers. There were eight cases whose FIGO

stage ranged from II to IV. Out of these, six were of endometrioid type and two were of non-endometrioid type. Both of the non-endometrioid tumors (squamous and mucinous) were in stage III. The other two non-endometrioid tumors were in stage I. The endometrioid cancer, which presented in stage IV, had omental deposits at the time of diagnosis. Paradoxically, this was a multiparous woman with more than three conceptions.

After a spate of studies on detection and significance of COX-2 expression in colon cancers, researchers explored the scope of investigation for non-colon solid cancers also. Detection of COX-2 was either by immunohistochemistry on solid tissues, in situ hybridization and northern blot analysis. In 2011, researchers from the Kings Institute of Preventive Medicine and Research, Chennai, published their observations that, COX-2 mRNA was overexpressed in breast cancer and lung cancer cell lines, while lower expression was observed in normal cell lines. They used the light cycler technology, which is now being considered the most precise method for the quantification of nucleic acid [(44)].

Shadab Mohammad, Hari Ram, Prem Narayan Gupta et al, examined the immunohistochemical overexpression of COX-2 in squamous cell carcinomas of the head and neck. They also sequentially evaluated the expression of COX-2 as the patients underwent radiation therapy. They observed that, the cases where COX-2 overexpression was observed did not respond effectively to radiation therapy [45].

Apart from these reports, observation of COX-2 overexpression in malignancies of uterine cervix, prostate and stomach are also published from India [46,47,48].

However, although western literature was available on study of overexpression of COX-2 in endometrial carcinomas, a thorough literature search performed on open search engines as well as medical databases did not reveal any publications from India.

As discussed in the review of literature, there are many articles on the study of COX-2 expression in tissues which differed in their assessment of the

immunohistochemical staining. For instance, Fowler JM, Ramirez N, Cohn D et al used a simple three tier scoring system which was more biased towards the intensity of cytoplasmic staining. The percentage of expression was kept a constant at 50%. Nasir A, Boulware D, Kaisre HE, et al. adopted a semi quantitative system whereby the intensity of staining and the proportion of cells that stained for COX-2 were scored separately. The final score was a mathematical derivation, which included adding up of the various scores found in the same tumor. This is the most appropriate method that would provide an accurate assessment of COX-2 overexpression. However, the authors have restricted the assessment to one block only. If the entire tumor was completely sampled and this semi quantitative system was to be applied, we would probably be deriving the most accurate information on COX-2 overexpression. As we are restricting our study to the assessment of one most representative block only, we preferreed to adopt the method proposed by Erkanli S, Bolat F, Kayaselcuk F et al. Accordingly, we first scored the percentage of cells that were positive for COX-2 expression. This was followed by an assessment of the intensity of staining. Whenever a higher staining intensity involving more than 25% of cells was noted, the score for that intensity was recorded even if the tumor had a larger proportion of cells with a lesser intensity of staining.

In our study, 97% of the endometrial carcinomas were positive for COX-2 immunomarker, of which 78% of the endometrial carcinomas were overexpressive for COX-2. Tong et al, found overexpression of COX-2 in 73% of their cases. Their sample size was small (11 cases) and they detected COX-2 both by immunohistochemistry and by in situ hybridization methods. The detection rate by both the methods was comparable [(49)]. Nasir A, Boulware D, Kaiser H.E, et al, also observed a very similar incidence of overexpression of COX-2 in endometrial cancer (76%) in their study population. Thus, it is obvious that the rate of overexpression of COX-2 in Indian women is similar to those reported in the west [(6)].

We observed a 100% overexpression of COX-2 in all those endometrial cancers that presented in stage II and above. Gabriella Ferrandina, Francesco Legge, Franco O. Ranelletti et al, studied the correlation between the overexpression of COX-2 and its clinical significance in endometrial carcinomas. They found that the COX-2 overexpression was higher in endometrial carcinomas presenting in stage II and above (61%) when compared with those tumors limited to the uterine corpus [(51)]. Lambropoulou M, Alexiadis G, Limberis V et al, however, found a statistically significant association between COX-2 overexpression and FIGO stage (p=0.010). Our

p value is not significant (0.093). However, this could change with increased recruitment of cases [52].

Although, many studies are evolving on assessing the association between those tumors that overexpressed COX-2 and mortality, we could not generate information on this, as our institute does not have a holistic oncology unit.

Most of the tumors (60%) in our study had a histologic grade of I ***(Colour plates 1 and 2)***. Of these, 77% showed overexpression for COX-2. While 75% of grade II tumors showed overexpression for COX-2 ***(Colour plates 3 and 4)***, there was a 100% overexpression for COX-2 in those tumors whose histologic grade was III. All the grade III tumors were of the non-endometrioid type.

Although, the p value was not statistically significant, we feel the increasing expression with increasing grade of tumor deserves to be acknowledged. Serkan Erkanli, Filiz Bolat, Fazilet Kayaselcuk et al, also did not find any

statistically significant correlation between COX-2 overexpression and histologic grade of the tumor [60]. However, Lambropoulou M, Alexiadis G, Limberis V et al, found a statistically significant correlation between histologic grading and COX-2 overexpression [52]. Gabriella Ferrandina, Francesco Legge, Franco O. Ranelletti et al, found that there was an exponential increase in COX-2 overexpression with grade. The COX-2 overexpression increased from 13.6% in grade I to 41.7% in grade II carcinomas. 61% of grade III carcinomas were positive for overexpression. They have also observed that this overexpression is statistically significant [51].

Most of the studies in the literature, have analyzed the expression of COX-2 only in the conventional endometrioid carcinomas. In Ferrandina G, Legge F, Ranelletti F et al's study all of the non-endometrioid tumors were of serous type of which 60% were overexpressive for COX-2. L.C. Horn, S. Faber, K. Bilek and C. Leo reported that their study population (8 cases) was only squamous cell carcinomas of corpus uteri. They observed that all the cases displayed overexpression for COX-2 [8]. In our study, four of the study population (37 cases) is of non-endometrioid type and although, each

of them was of histologically different type, all of them were overexpressive for COX-2.

One of the non-endometrioid tumors was of the serous type. It occurred in a 54-year-old woman as postmenopausal bleeding. The tumor was polypoid and friable. It showed complex papillae lined by cells with grade III nuclei, invading the inner half of the myometrium. Psammoma bodies were seen focally. All the tumor cells were strongly immunoreactive for COX-2 with a diffuse cytoplasmic staining ***(Colour plate 5).***

Clear cell carcinoma was diagnosed in a 56-year-old female whose presentation was postmenopausal bleeding and lower abdominal pain. The entire uterine cavity, which was distended, was filled by a pale brown friable growth, that extended into the lower uterine segment and also infiltrated the myometrium. This tumor showed varying patterns such as glandular, tubulocystic, tubulopapillary and solid. Large cells with clear cytoplasm dominated the histological picture. A few hobnail cells were also seen. 75-100% of tumor cells were immunoreactive for COX-2. However, the intensity was diffuse and weak ***(Colour plate 6).***

Mucinous carcinoma occurred in a 60-year-old woman who was admitted with complaints of pain abdomen for four months and brownish discharge for four days. The uterine cavity, which was markedly dilated, was cystic in consistency and contained about 200 ml of brownish viscous fluid. The wall was trabeculated with multiple small polyps on it. These polyps were soft and homogenous and appeared to be stuck on the endometrial surface. Histologically, the tumor showed a predominant tubular pattern. These were lined by mucinous epithelium with high-grade nuclei. In some areas the tumor cells were poorly differentiated and pleomorphic. This patient also had a high grade tumor confined to the fallopian tube. This could be a synchronous lesion. All the tumor cells display strong and diffuse cytoplasmic staining for COX-2 ***(Colour plate 7)*.**

Squamous cell carcinoma of the uterine corpus was reported in a 70-year-old woman with postmenopausal bleeding. The tumor was polypoidal with fine papillary excrescences. Histologically, the tumor was composed of malignant squamous cells arranged in sheets, nests and finger like infiltrating patterns with numerous areas of necrosis. Keratin pearls were conspicuous in the viable areas. The tumor was found to extend up to the middle third of the right fallopian tube. 50-75% of the tumor cells were

immunoreactive to COX-2, but displayed diffuse and weak cytoplasmic staining only ***(Colour plate 8).*** The pattern of immunostaining observed in our study is very unlike those observed by Horn LC, Faber S, Bilek K and Leo C,, the latter had observed a diffuse and strong immunostaining in all the squamous cell carcinomas of the endometrium they had studied.

78% of those cases where the tumor had invasion greater than half of the myometrium, were found to be overexpressive for COX-2. This increased expression of COX-2 in such tumors has been observed in almost all of the studies published in literature [7,51.52,53,54]. While Gabriella Ferrandina, Francesco Legge, Franco O. Ranelletti et al [51], and Lambropoulou M, Alexiadis G, Limberis V et al [52], observed the association to be statistically significant, Jeffery M. Fowler, Nilsa Ramirez, David E. Cohn et al, did not find it statistically significant [54]. However, Jeffery M. Fowler, Nilsa Ramirez, David E. Cohn et al observed that on a multivariate analysis, non-endometrioid histology and a myometrial invasion of greater than 50% were predictive of advanced stage at presentation [54].

We observed that, the overexpression of COX-2 was almost the same between premenopausal women (80%) and postmenopausal women (78%). Thus, there is no correlation between the menopausal status and COX-2 overexpression. There is no published information evaluating this parameter, although many endometrial carcinomas do occur in premenopausal women too.

We did not find any significant difference in patients with endometrial carcinoma overexpressing COX-2 based on their parity. This parameter too, has not been evaluated in the literature.

SUMMARY:

- During the study period (1st January, 2007 to 31st December 2013), the Histopathology laboratory had received a total of 35,449 biopsy specimens of which 3406 (9.6%) were reported as malignant.

- Of these, 54 (1.6%) were reported as endometrial carcinoma.

- The median age at presentation of patients in our study population (37 cases) was 59 years and most of the patients were in their 6th and 7th decades of life.

- Endometrioid carcinoma accounted for 33 of the 37 cases. The rest were non-endometrioid carcinoma.

- 78% of the patients in our study population presented with a FIGO stage I disease.

- 97% of the endometrial carcinomas showed expression of the COX-2 immunomarker.

- Overexpression for the COX-2 immunomarker was observed in 78% of the cases.

- Among the endometrioid type of endometrial cancers, 73% of cases overexpressed COX-2, whereas all of the non-endometrioid type carcinomas (100%) were observed to overexpress COX-2.

- The occurrence of overexpression of the COX-2 immunomarker was analogous in grade I and grade II endometrial carcinomas (75-78%), while all of the cases that presented with a FIGO grade III lesion (100%) overexpressed COX-2.

- COX-2 overexpression did not show any significant difference between tumors with invasion up to less than or more than half of the myometrial thickness.

- There was no relationship between COX-2 overexpression and parameters such as parity and menopausal status.

CONCLUSIONS:

The age group, the menopausal status, histologic grade, FIGO stage at presentation and the histologic type of endometrial carcinomas in our study was similar to those described in literature. All but one of the endometrial carcinomas expressed COX-2. Overexpression was consistently observed in all patients who presented in stage II and above. All of the non-endometrioid carcinomas were overexpressive for COX-2. Although, we did not obtain a statistical significance, yet we consider that the observations are significant to be reported. A study of a larger cohort of population might elucidate statistically significant results.

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MASTER CHART

LAB NO	AGE	PARITY	MENSTRUAL STATUS	STAGE	GRADE	TYPE	MYOMETRIAL INVASION	SCORE OF % OF CELLS +ve	INTENSITY SCORE	TOTAL SCORE	OVER EXPRESSION
S350/13	43	1	2	3	1	1	1	4	2	6	1
S1002/13	77	1	1	1	1	1	1	4	1	5	1
S1312/13	42	1	1	1	2	1	1	4	1	5	1
S1453/13	55	1	1	1	2	1	2	4	1	5	1
S1465/13	50	1	1	2	2	1	1	3	3	6	1
s1630/13	70	1	1	1	2	1	1	2	1	3	**2**
S1907/13	80	2	1	2	1	1	1	4	3	7	1
S3251/13	59	2	1	1	2	1	2	4	3	7	1
S3610/13	55	1	1	1	1	1	1	4	2	6	1
S3619/13	44	1	2	1	1	1	1	2	1	3	**2**
S3893/13	62	1	1	1	2	1	2	4	2	6	1
S4364/13	60	2	1	1	1	1	1	4	3	7	1
S4570/13	64	1	1	1	2	1	2	3	1	4	1
S4732/13	61	1	1	1	2	1	1	4	3	7	1
S394/12	63	2	1	4	2	1	1	3	1	4	1
S1261/12	73	2	1	1	1	1	1	4	1	5	1

Parity: 1 = ≤3 & 2 =>3; **Menstrual status:** 1 = Postmenopausal & 2 = Premenopausal; **Type:** 1 = Endometrioid carcinoma & 2 = non-endometrioid carcinoma;

Myometrial invasion: 1 = ≤1/2 & 2 = >1/2; **Overexpression:** 1 = positive & 2 = negative

MASTER CHART

LAB NO	AGE	PARITY	MENSTRUAL STATUS	STAGE	GRADE	TYPE	MYOMETRIAL INVASION	SCORE OF % OF CELLS +ve	INTENSITY SCORE	TOTAL SCORE	OVER EXPRESSION
S2113/12	66	2	1	1	2	1	1	2	1	3	**2**
S4078/12	67	1	1	1	1	1	1	4	2	6	1
S4328/12	60	1	1	1	1	1	1	4	1	5	1
S2602/11	60	2	1	3	3	2	2	4	3	7	1
S3996/11	59	1	1	1	1	1	1	2	1	3	**2**
S4579/11	58	1	1	1	1	1	1	4	2	6	1
S4890/11	70	2	1	3	2	2	2	3	1	4	1
S3583/10	24	1	2	2	1	1	1	4	3	7	1
S3706/10	52	1	1	1	1	1	1	4	2	6	1
S3898/10	50	1	1	1	1	1	2	1	1	2	**2**
S4393/10	60	1	1	1	1	1	2	1	1	2	**2**
S4597/10	27	1	2	1	1	1	1	4	2	6	1
S4714/10	58	2	1	1	1	1	1	3	2	5	**1**
S1116/09	53	1	1	1	3	2	2	4	1	5	1
S1014/08	54	2	1	1	3	2	1	4	3	7	1
S1355/08	29	1	2	1	1	1	1	4	2	6	1
S2627/08	57	1	1	1	1	1	1	3	1	4	1

Parity: 1 = ≤3 & 2 =>3; **Menstrual status:** 1 = Postmenopausal & 2 = Premenopausal; **Type:** 1 = Endometrioid carcinoma & 2 = non-endometrioid carcinoma;

Myometrial invasion: 1 = ≤1/2 & 2 = >1/2; **Overexpression:** 1 = positive & 2 = negative

MASTER CHART

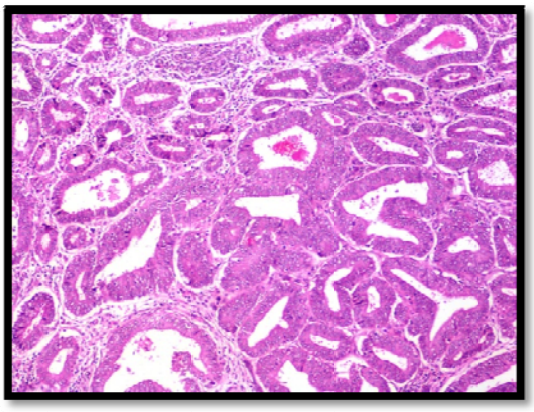
LAB NO	AGE	PARITY	MENSTRUAL STATUS	STAGE	GRADE	TYPE	MYOMETRIAL INVASION	SCORE OF % OF CELLS +ve	INTENSITY SCORE	TOTAL SCORE	OVER EXPRESSION
S3326/08	55	1	1	3	1	1	1	4	3	7	1
S165/07	70	1	1	1	1	1	1	4	2	6	1
S520/07	59	2	1	1	2	1	1	2	1	3	**2**
S1224/07	72	2	1	1	1	1	1	0	0	0	**2**

Parity: 1 = ≤3 & 2 =>3; **Menstrual status:** 1 = Postmenopausal & 2 = Premenopausal; **Type:** 1 = Endometrioid carcinoma & 2 = non-endometrioid carcinoma;

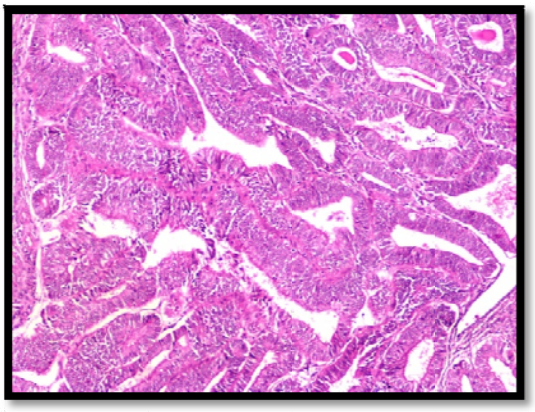
Myometrial invasion: 1 = ≤1/2 & 2 = >1/2; **Overexpression:** 1 = positive & 2 = negative

Immunoreactivity for COX – 2 in a histologic grade – 1 endometrioid adenocarcinoma

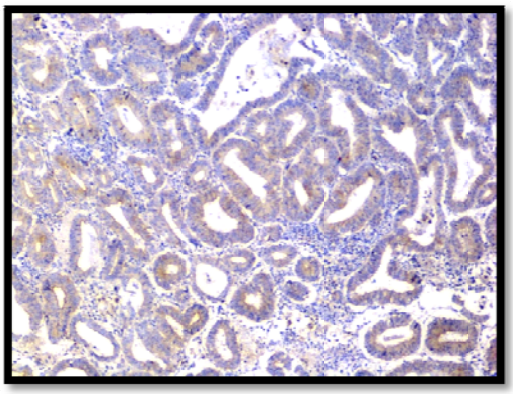
Colour plate 1



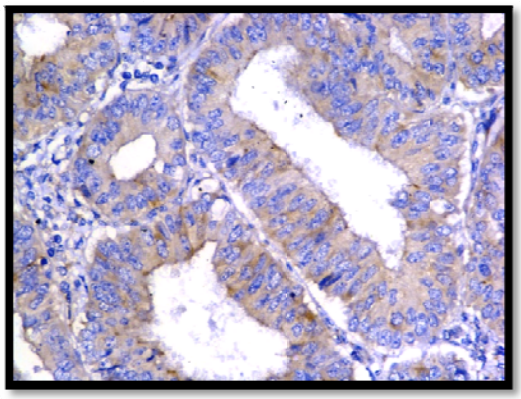
Tumor showing neoplastic endometrial glands arranged back-to-back with architectural abnormalities. H&E; x 100



The tumor cells are large, with nuclei displaying loss of polarity and nucleoli. H&E; x 450



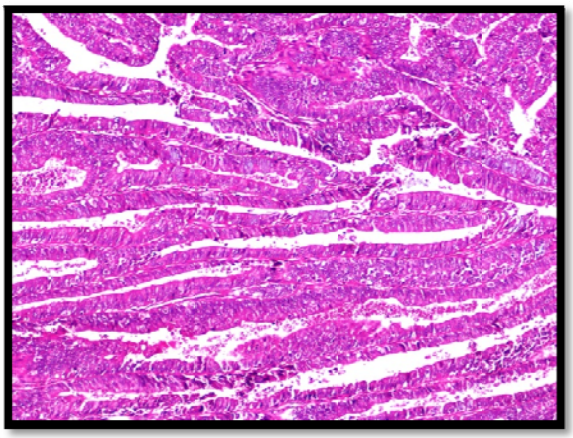
>75% of the tumor cells are immunoreactive for COX-2.



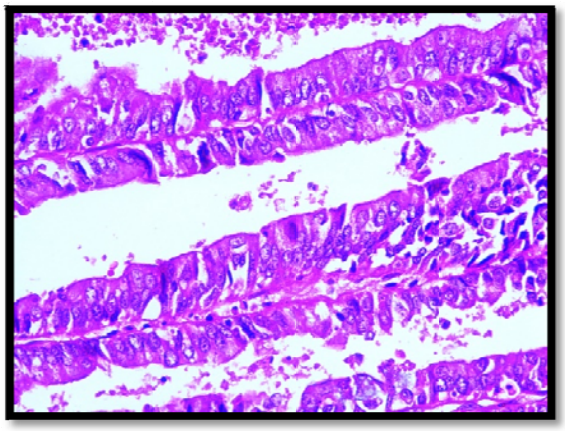
The tumor cells display diffuse and weak cytoplasmic staining for COX-2.

Immunoreactivity for COX – 2 in a villoglandular variant of endometrioid adenocarcinoma

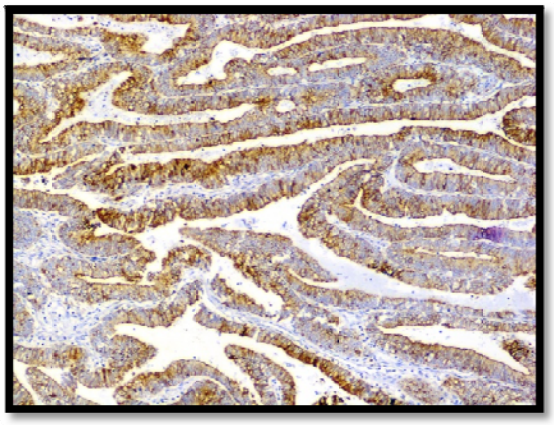
Colour plate 2



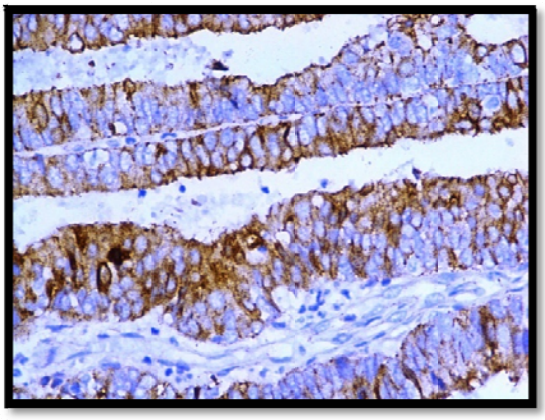
Tumor composed of long, slender papillae. H&E; x 100



The papillae are narrow and the cells display Grade 1 nuclear features. H&E; x 450



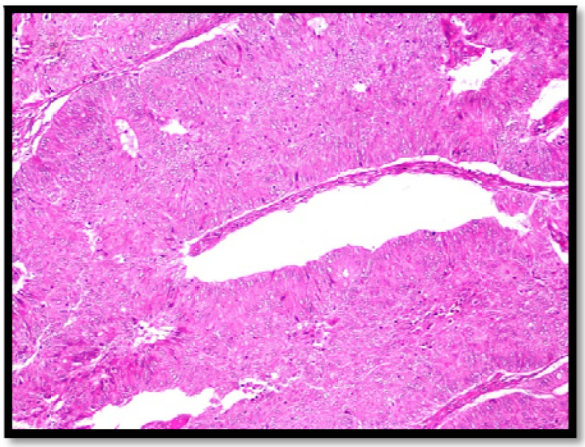
>75% of the tumor cells are immunoreactive for COX-2.



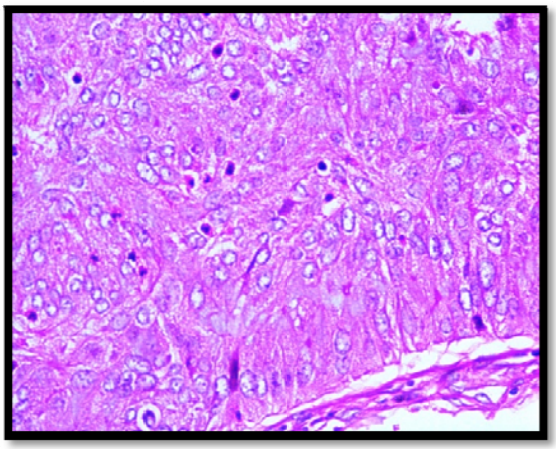
The tumor cells display a moderate to strong granular cytoplasmic staining for COX-2.

Immunoreactivity for COX – 2 in a histologic grade 2 endometrioid adenocarcinoma

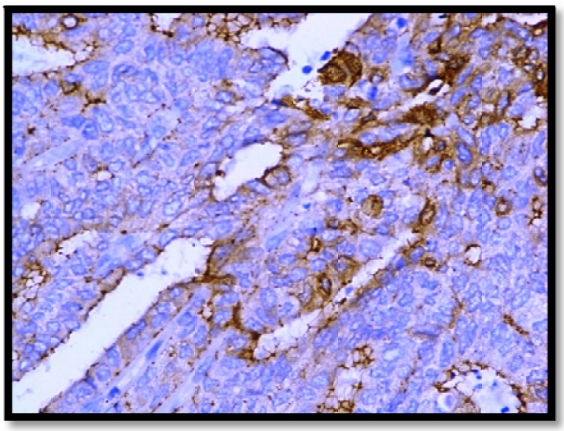
Colour plate 3



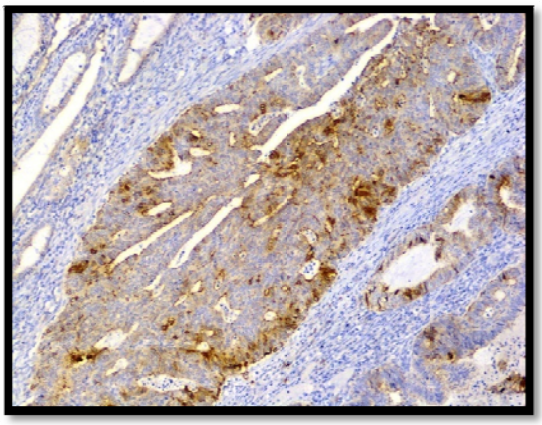
Tumor showing solid areas which are non-squamoid and comprise <50% of the total area. H&E; x 100



The nuclei are round to oval, with evenly distributed chromatin and inconspicuous nucleoli. H&E;x450



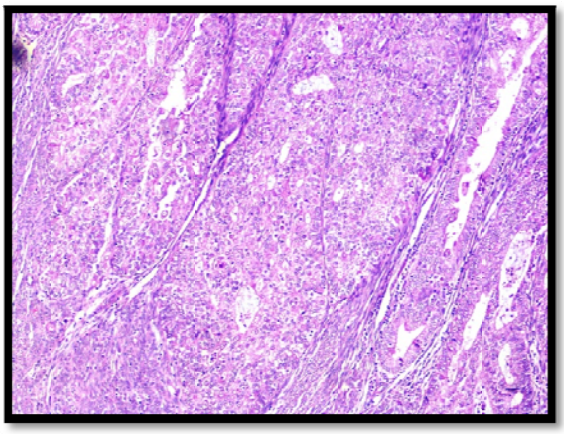
50% to 75% of the tumor cells are immunoreactive for COX-2.



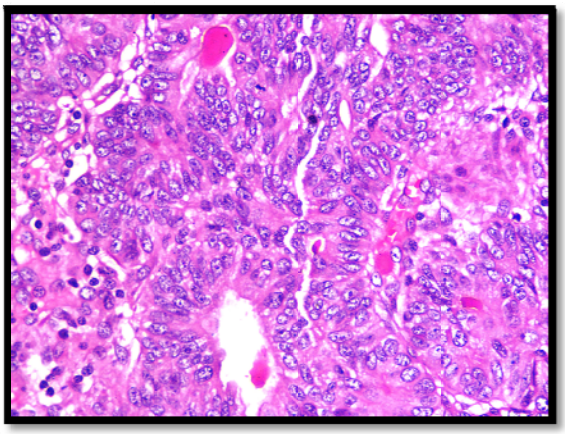
The tumor cells display a moderate to strong granular cytoplasmic staining for COX-2.

Immunoreactivity for COX – 2 in a histologic grade 2 endometrioid adenocarcinoma

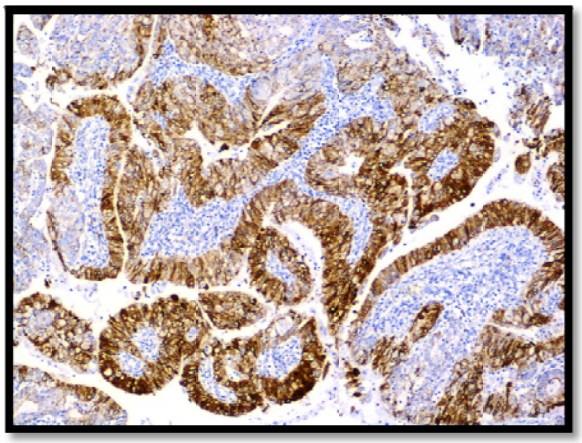
Colour plate 4



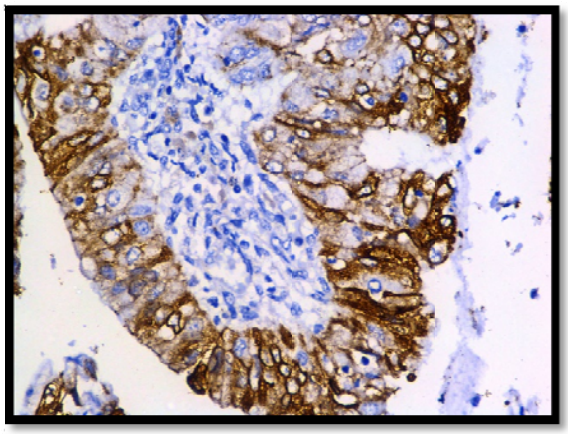
Tumor showing solid areas which are non-squamoid and comprise <50% of the total area. H&E; x 100



Tumor cells display irregular oval nuclei with chromatin clumping and moderate sized nucleoli.H&E;x 450



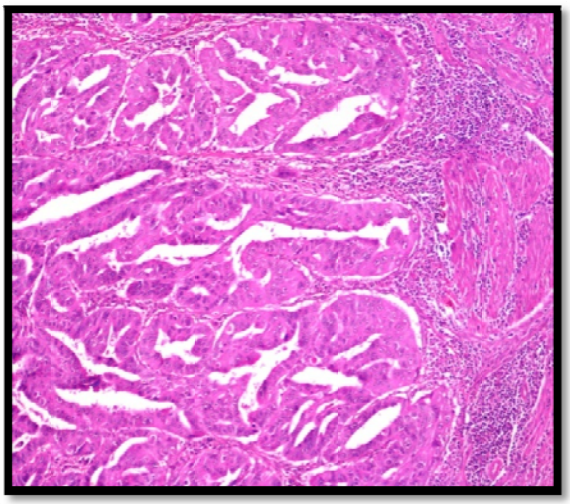
>75% of the tumor cells are immunoreactive for COX-2.



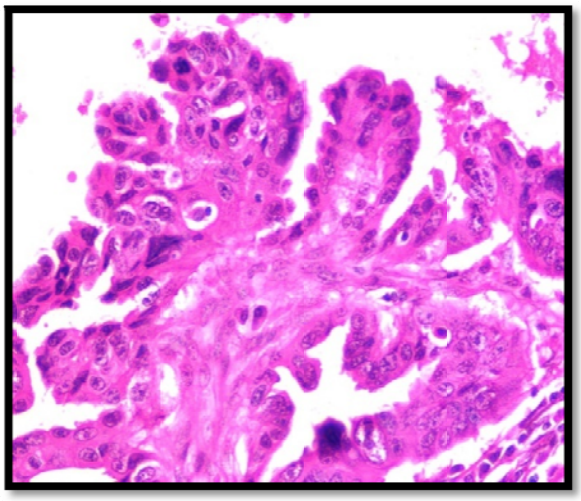
The tumor cells display strong and diffuse cytoplasmic staining for COX-2.

COX – 2 in serous adenocarcinoma of uterus

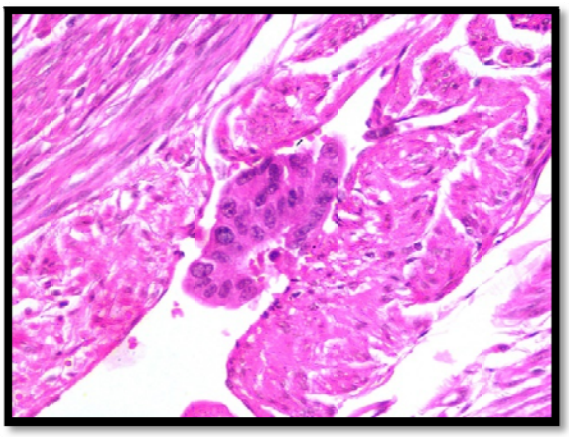
Colour plate 5



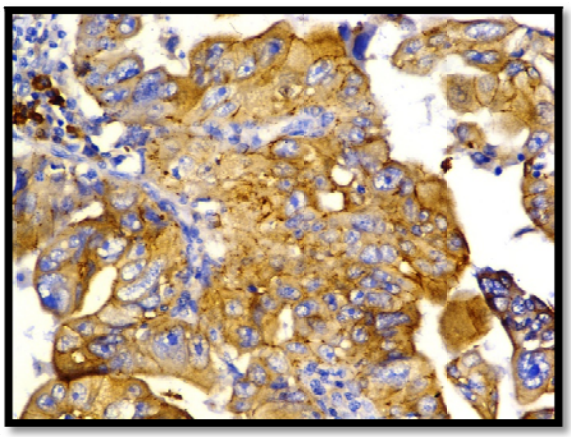
Tumor showing a marked, complex, papillary architecture. H&E; x 100



The papillae are broad and show, secondary and tertiary papillary processes. The nuclei are large, pleomorphic and hyperchromatic. H&E; x 450



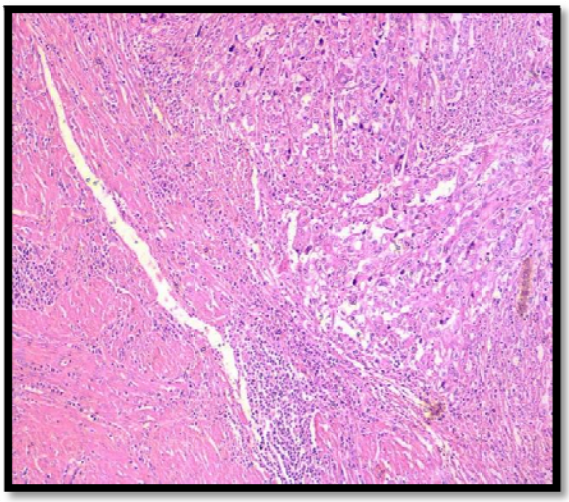
Tumor cells are seen invading the myometrium. H&E; x 450



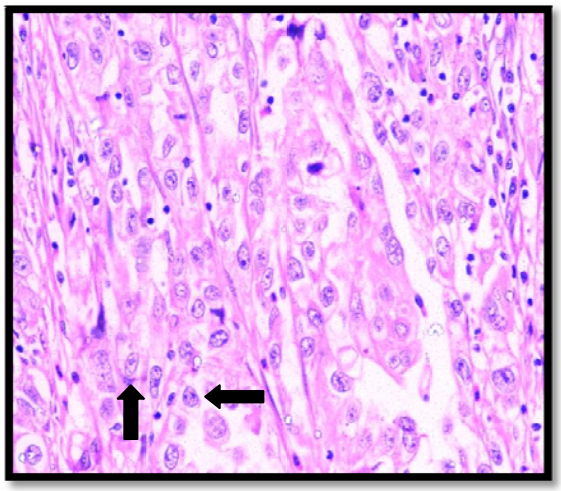
All of the tumor cells show a strong and diffuse cytoplasmic staining for COX-2.

COX – 2 in clear cell adenocarcinoma of uterus

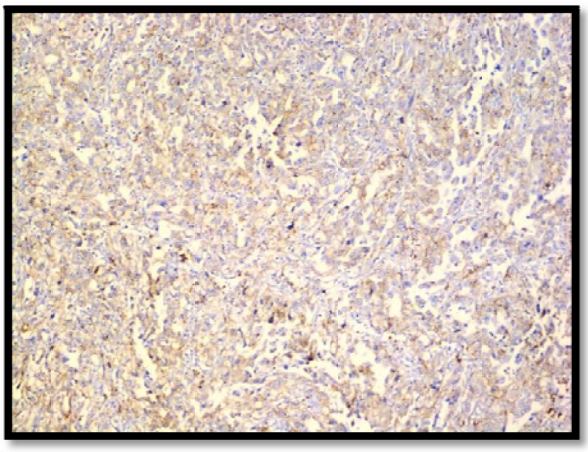
Colour plate 6



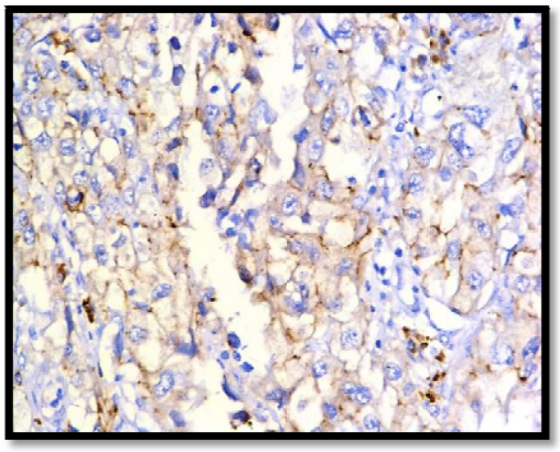
Tumor showing glandular and tubular-cystic patterns. H&E; x 100



Cells with abundant clear cytoplasm (up arrow) and hobnail cells (left arrow) projecting into the lumen are seen. H&E; x 450



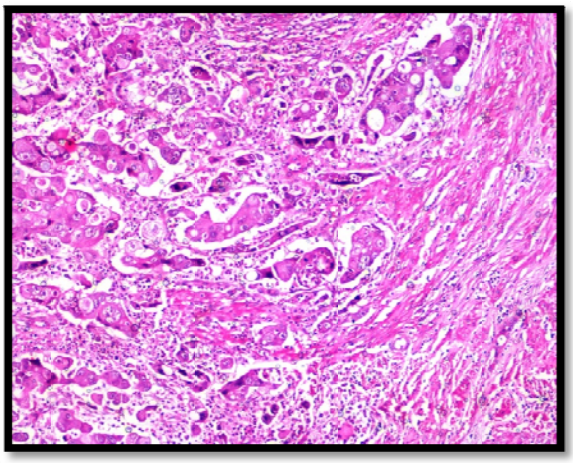
>75% of the tumor cells are immunoreactive for COX-2.



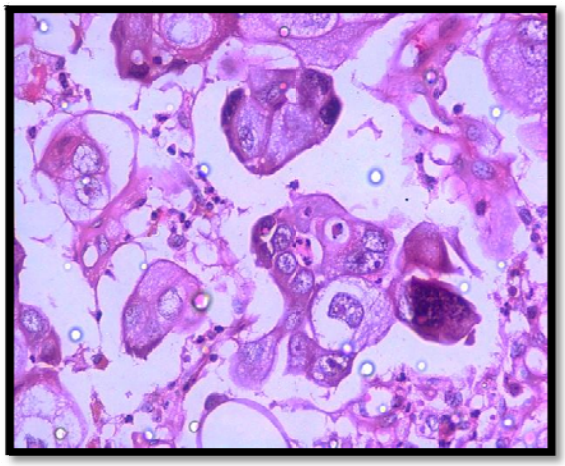
The tumor cells display diffuse and weak cytoplasmic staining for COX-2.

COX – 2 in mucinous adenocarcinoma of uterus

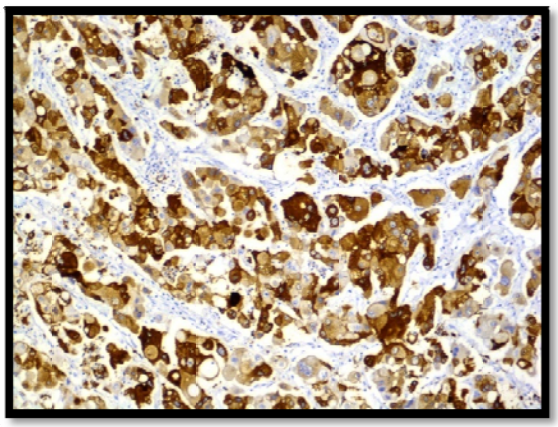
Colour plate 7



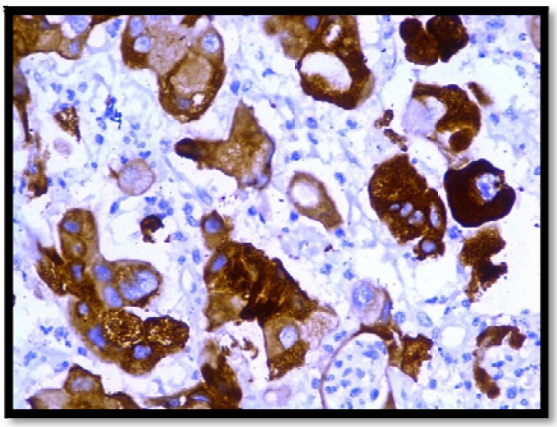
Tumor showing tubulo-cystic and solid patterns, invading the myometrium. H&E; x 100



Malignant cells with abundant intra-cytoplasmic and extracellular mucin. H&E; x 450



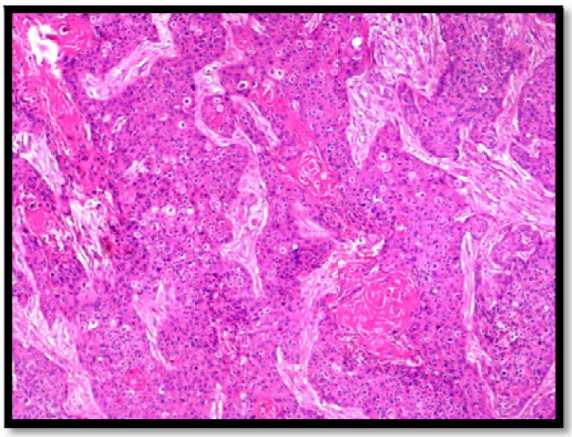
>75% of the tumor cells are immunoreactive for COX-2.



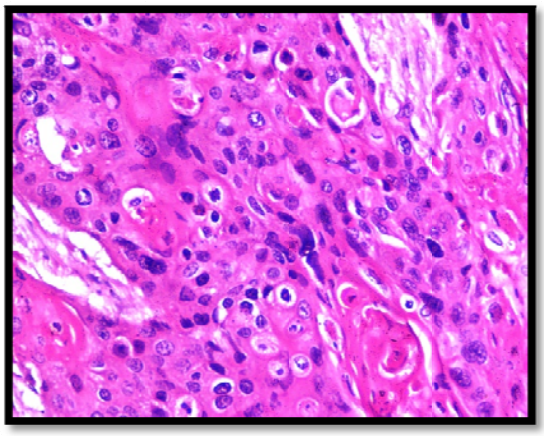
The tumor cells display strong and diffuse cytoplasmic staining for COX-2.

COX – 2 in squamous cell carcinoma of uterus

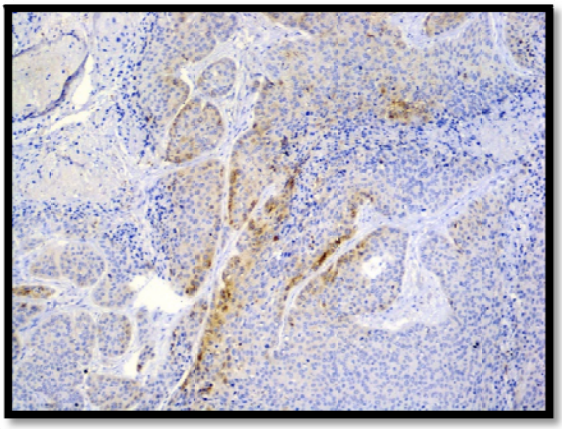
Colour plate 8



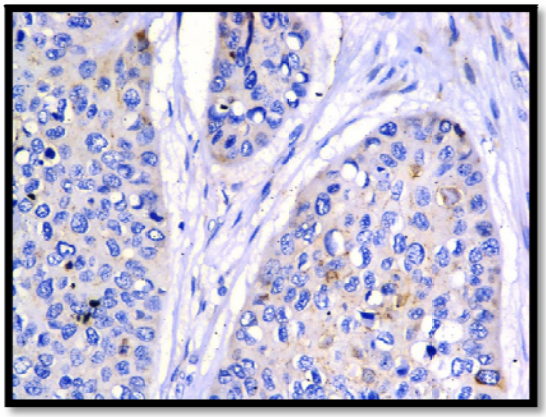
Tumor showing a tentacular / finger – like infiltrating pattern of growth. H&E; x 100



Tumor cells which display hyper chromatic nuclei are also associated with the presence of keratin pearls H&E; x 450



50% to 75% of the tumor cells show immunoreactivity to COX-2.



The tumor cells display diffuse and weak cytoplasmic staining for COX-2.